STAIN TECHNOLOGY

VOLUME II JANUARY, 1936 NUMBER 1

AN ENLARGEMENT OF STAIN TECHNOLOGY

It is a happy coincidence that at the end of STAIN TECHNOLOGY'S first decade we are able to announce, after a year's preparation, the expansion of the section entitled "LABORATORY HINTS FROM THE LITERATURE" into a much larger department under the charge of one man as abstract editor. The enlargement of this section is to be such as to constitute virtually a new feature for the journal. This idea has been under consideration for some time and announcement of the plans were made in SCIENCE early this year. The response to this announcement was very much greater than we anticipated. We have not only found the Commission members ready with enthusiastic support of the plan, but have received so many offers for assistance from those not previously associated with the Commission that this new undertaking now has a fair-sized organization of abstracters behind it. The editors hereby extend their thanks to all those who have offered to assist. The result of this collaboration should certainly be a better and larger department of "LABORATORY HINTS". It is hoped that this venture will find adequate reception among our readers and biologists at large.

The policy in this section of the journal has always been to furnish the readers with abstracts that are *usable* in the laboratory, and this same plan will be followed in the future whenever possible. Accordingly, the abstracts published in STAIN TECHNOLOGY ordinarily give the technic in sufficient detail so that it can be followed without consulting the original article. The exceptions to this rule are those papers where a great variety of procedures is described, and those which deal with dyes in general rather than with technic. Altho our policy will be followed wherever possible, exceptions may be more frequent in the future than in the past, because the field covered is no longer to be limited to a few subjects. We plan, in fact, to include any line that might be of interest to users of the microscope in general. Naturally, special attention will be paid in the future, as it has always been in the past, to dyes in all their biological applications.

At the present time this service covers some 160 journals issued in 21 different countries. These journals have been assigned to those who have offered to help in the work; and the large number of abstracts secured for the present number shows that the abstracters are actually coöperating. The present number contains 60 abstracts and represents the work of 10 abstracters. We wish here to express our appreciation not only to the abstracters who have actually contributed to this number, but also to all the other collaborators (there are nearly 50 in all) whose assistance has made it possible to undertake the project.

—J. A. DE TOMASI, *Abstract Editor*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the October number of this journal.

STAINS CERTIFIED SEPTEMBER 1, 1935 TO NOV. 30, 1935*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Crystal violet	LC 9	92%	As bacteriological, histological and cytological stain	Sept. 5, 1935
Safranin O	LS 5	87%	As cytological and histological stain	Sept. 10, 1935
Indigo carmine	NI 5	91%	As histological stain	Oct. 21, 1935
Basic fuchsin	CF 15	91%	For general staining and in the Endo medium	Nov. 21, 1935

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

SUGGESTED COUNTERSTAINS FOR DAVENPORT REDUCED SILVER PREPARATIONS OF PERIPHERAL NERVES

JAMES O. FOLEY, *Department of Anatomy, University of Alabama, University, Ala.*

ABSTRACT.—**Peripheral nerves which have been fixed in a mixture of formaldehyde and acetic acid and stained according to the method of Davenport can be successfully counterstained for demonstration of myelin sheaths and stroma. After mounted sections have been silvered, reduced and toned, the coating of nitrocellulose is removed by passing thru two changes of acetone. Following brief washes in 100, 95, 85 and 75% alcohols they are stained in an acidified aqueous solution of azo carmine for 30 to 60 minutes. Excess azo carmine is extracted with anilin alcohol followed by acetic alcohol after which the sections are mordanted for 15 to 60 minutes in a 5% aqueous solution of phosphotungstic acid. Without washing they are transferred to a stain mixture of either anilin blue and orange G (acidified) or light green and orange G (acidified) where they remain from 1 to 5 hours. After destaining in 95% alcohol and dehydration in absolute alcohol the sections are mounted in dammar. Result: axons stain black; sheath and fibroblast nuclei, red; myelin sheaths, orange; and connective tissue, blue or green. When the counterstains are applied to ganglia, cytological details of individual cells are demonstrated.**

INTRODUCTION

When most silver methods are applied to peripheral nerves, axis cylinders stain selectively; consequently, sheaths and supporting tissues are incompletely demonstrated. Large myelinated fibers can be identified readily in such preparations, since the big axons are surrounded by large clear areas (unstained sheaths). Small myelinated fibers, however, can be recognized less easily, since the axons are reduced in diameter and the enveloping clear zones are decreased proportionately. In fact, differentiation between the smallest myelinated and the largest unmyelinated fibers frequently is impossible. To add to the confusion, clumps of chromatin in sheath nuclei occasionally simulate unmyelinated axons. These inadequacies of silver when used alone suggest the need for counterstains which will show clearly both sheath and stroma, and thus aid in separation and classification of fibers according to type. Helpful in this direction are variations of Heidenhain's modification of the Mallory triple

stain as described by Romeis[1] which we have used in conjunction with the Davenport[2] silver technic.

A. Stock Solutions of Stains

Stains and Reagents Employed

1. AZOCARMINE MIXTURE

Azocarmine (Grübler, dye content not stated)	1.0 g.
Glacial acetic acid (Merck, C. P. 99%)	4.0 cc.
Distilled water	100.0 cc.

2. ANILIN BLUE ORANGE G MIXTURE

Anilin blue (Grübler, dye content not stated)	0.5 g.
Orange G (Coleman and Bell Co., Certified 82%, representing 1.64 g. actual dye)	2.0 g.
Glacial acetic acid (Merck, C. P. 99%)	1.0 cc.
Distilled water	100.0 cc.

3. LIGHT GREEN ORANGE G MIXTURE

Light green (Eberbach & Sons Co., dye content not stated)	0.5 g.
Orange G (Coleman and Bell Co., Certified 82%, representing 1.64 g. actual dye)	2.0 g.
Glacial acetic acid (Merck, C. P. 99%)	1.0 cc.
Distilled water	100.0 cc.

4. NOTE: Stock solution No. 1 is used full strength. 10 cc. of stock solution No. 2 or No. 3 are diluted with 40 cc. of distilled water before using.

B. Stock Reagents

1. Acetone (Baker's analyzed, C. P.)
2. Anilin oil (Coleman and Bell Co., C. P.)
3. Dammar (Coleman and Bell Co.)
4. Formaldehyde (Merck's blue label, 37%)
5. Glacial acetic acid (Merck, C. P., 99%)
6. Gold chloride (Merck, C. P.)
7. Low viscosity nitrocellulose (Hercules Powder Co.)
8. Paraffin (Leitz, white filtered, M. P. 60–62° F.)
9. Petroleum ether (Coleman and Bell Co., b. p. 40–60° C.)
10. Phosphotungstic acid (Merck)
11. Pyrogallic acid (Mallinckrodt, C. P.)
12. Silver nitrate (Merck's reagent grade or Coleman and Bell Co., C. P.)
13. Sodium thiosulfate
14. Xylol (Coleman and Bell Co.)

---

[1]Romeis, B. Taschenbuch der Mikroskopischen Technik. R. Oldenbourg, München. pp. 365–6. 1928.

[2]Davenport, H. A. Staining nerve fibers in mounted sections with alcoholic silver nitrate solution. Arch. Neur. and Psych., **24**, 690–5. 1930.

Procedure

A. Technic of Staining with Silver.—Stretches of the glossopharyngeal and vagus nerves of the cat were fixed in a mixture of formaldehyde and acetic acid (formaldehyde 25 cc., glacial acetic acid 5 cc., and distilled water 70 cc.), dehydrated in graded ethyl alcohols, cleared in xylol and embedded in paraffin. Sections 5 microns thick were silvered, reduced and toned according to the method described by Davenport.[3]

After the sections had been toned in gold chloride, treated for 5 minutes with a 5% aqueous solution of sodium thiosulfate and thoroly washed in tap water, the coating of nitrocellulose was removed by passing the slide with constant agitation thru two changes of acetone. Finally, the tissues were prepared for the counterstain by brief washes in 100, 95, 85, and 75% alcohols.

B. Technic of Counterstaining.—(All procedures at room temperature.) 1) From 75% alcohol to azocarmine; 2) Azocarmine, *30 minutes to one hour;* 3) Destain in anilin alcohol (95% alcohol 1000 cc., anilin oil 1 cc.) *until nuclei are clearly differentiated.* (Process may be facilitated by passing slide back and forth from 95% to 75% alcohol.) 4) Acetic alcohol (95% alcohol 1000 cc., and glacial acetic acid 1 cc.), *1 to 5 minutes;* 5) Phosphotungstic acid (5% aqueous solution), *15 minutes to 1 hour;* 6) Either anilin blue orange G mixture, or light green orange G mixture, *1 to 5 hours;* 7) Destain in 95% alcohol, *until tissue elements (other than axons) are clearly differentiated.* (Process may be facilitated by passing slide back and forth from 95% to 75% alcohol.) 8) Absolute alcohol, two changes, *1 to 3 minutes each;* 9) Xylol, four changes, *several minutes each;* 10) Mount in dammar which has been extracted with cold petroleum ether, evaporated to dryness, and dissolved in xylol.

Results

Axis cylinders are stained black, sheath and fibroblast nuclei red; myelin sheaths orange; and connective tissue blue or green, the latter depending upon whether anilin blue or light green is used (Figs. 1 and 2). If too much azocarmine is removed during the destaining process, the nuclei and the sheaths will be yellow instead of red and orange, respectively.

---

[3]Thru the courtesy of Dr. Davenport we have employed his method with certain modifications which he has suggested (see footnote 2) and which we have found advantageous. These alterations of the original technic are: a) the use of a 20% instead of a 10% alcoholic silver bath; b) an 8% instead of a 5% concentration of pyrogallic acid; and c) a 4% solution of nitrocellulose instead of a 2% solution of celloidin.

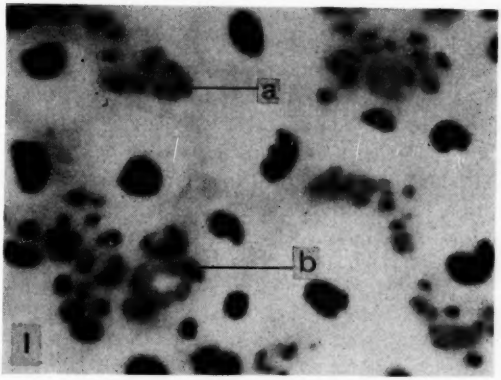
PLATE I, EXPLANATION OF FIGURES

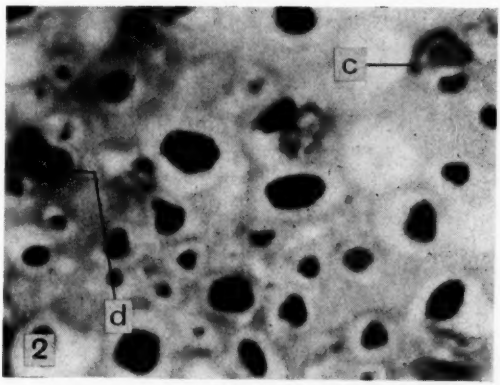
Photomicrographs of sections of the vagus and glossopharyngeal nerves of the cat.

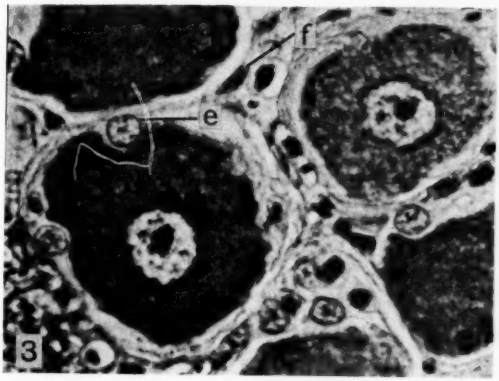
1. A cross section of the vagus below the nodose ganglion (Davenport silver; azocarmine, anilin blue and orange G counterstain; × 3000). Note the clump of unmyelinated fibers (a). In full color the tissue core of (a) is blue or purple whereas the core of (b), a nucleus, is red; the myelin sheaths are orange, while the intervening collagenous tissues are blue. It should be observed that the groups of black unmyelinated fibers are embedded in collagenous tissue.

2. A cross section of the glossopharyngeal below the petrous ganglion (Davenport silver; azocarmine, light green and orange G counterstain; × 3200). All axons in the field are myelinated. Observe the range in size of the axons. Each is surrounded by an orange myelin sheath (light gray and white in photograph) and is separated from adjacent fibers by green collagenous tissue (dark gray in photograph). In contrast to the vagus nerve (Fig. 1) there are no clumps of unmyelinated fibers. Note the sheath nuclei, (c, d). These stain red and each is related to a small myelinated fiber.

3. A cross section thru the nodose ganglion of the vagus (Davenport silver; azocarmine, anilin blue and orange G counterstain; × 800). Attention is directed to the sharpness with which the neuro-fibrillae and nucleoli of the cells are outlined (particularly in the cell at the upper right of the photograph). Note the fibroblast nucleus (f) of the connective tissue capsule of the ganglion cell and the nucleus (e) of the sheath of Schwann cell. Several myelinated fibers are clearly shown between the two nucleated cells.


a
b
1


c
d
2


f
e
3

When the counterstain is applied to ganglia, myelin sheaths of nerve fibers, sheath nuclei and stroma are stained as above. In addition, the finer cytological details of the individual ganglion cells are demonstrated, the neurofibrillae of the cells being black, the hyaloplasm orange or yellow, and the nucleolus red. Likewise, the nuclei of the connective tissue capsule of each cell and those of the Schwann cell capsule are fully differentiated (Fig. 3). Since by the methods outlined nucleoli of cells can be stained, one should be able to make counts of ganglion cells in nerves that have been silvered previously for axon studies.

LACTO-PHENOL PREPARATIONS

W. E. Maneval, *University of Missouri, Columbia, Missouri*

Abstract.—**More or less permanent mounts of fungi, algae, root tips, epidermis, germinating spores, and other small objects may be made readily by transferring the material to Amann's lacto-phenol containing anilin blue, W. S. or acid fuchsin, used singly or mixed. The addition of 20 to 25% of glacial acetic acid to these mixtures is frequently advantageous; or material may be stained with various dyes—acid fuchsin, anilin blue, W. S. (cotton blue), rose bengal, phloxine, hematoxylin—in aqueous solutions containing 5% of phenol, and then mounted in lacto-phenol, 50% glycerin or phenol-glycerin, depending on the dye used. The phenol solutions of acid fuchsin and anilin blue are acidified with acetic acid and those of rose bengal and phloxine are made slightly alkaline with ammonium hydroxide. The addition of ferric chloride to acid fuchsin or acidified hematoxylin may improve staining. Fixation may be preferable but may be omitted, especially with fungi. Formulae for the mounting media and ten staining mixtures are given.**

Lacto-phenol-dye mixtures have long been used for temporary mounts, especially of fungi. Much less cotton blue than is recommended by Linder[1] is often preferable. Substitution of another acid dye (especially acid fuchsin) for cotton blue (syn. anilin blue, W. S.), or using two dyes mixed together is frequently advantageous. The addition of 20 to 25% of glacial acetic acid makes staining more rapid and generally not as deep as without the acid. Fungi transferred to this mounting medium may be studied immediately or the preparations may be kept for a year or longer. In making such mounts many variations are possible. The formulae for the various stains and mounting media are given below. The following dyes were used: Acid fuchsin, cotton blue (anilin blue, W. S.) and so-called "Magdala red", Coleman and Bell (the last named being actually phloxine); rose bengal, Grübler; hematoxylin, Coleman and Bell, C. P., Certification No. FH-5.

Formulae

1. *a. Lacto-phenol, Amann's:* phenol, 20 g. or cc.; lactic acid, 20 cc.; glycerin, 40 cc.; water, 20 cc.
   b. Phenol-glycerin: phenol, 20 g. or cc.; glycerin, 40 cc.; water, 40 cc.

[1]Linder, David H. An ideal mounting medium for mycologists. Science, **70**, 430. 1929.

2. *Lacto-phenol-dye (1% aqueous) mixtures.*
   a. Lacto-phenol, 100 cc.; cotton blue, 1 to 5 cc.; glacial acetic acid, 0 or 20 cc.
   b. Lacto-phenol, 100 cc.; acid fuchsin, 1 to 5 cc.; glacial acetic acid, 0 or 20 cc.
   c. Lacto-phenol, 100 cc.; acid fuchsin and anilin blue, W. S., 1 to 3 cc. each; glacial acetic acid, 0 or 20 cc.

For algae, fungi, root tips, epidermis, *Elodea* leaves, etc. Mount in lacto-phenol or lacto-phenol plus 20% of glacial acetic acid.

3. *Phenol (5% aqueous) dye (1% aqueous) mixtures.*
   a. Phenol, 15 cc.; anilin blue, W. S., 0.5 to 1 cc.; glacial acetic acid, 4 cc.
   b. Phenol, 15 cc.; acid fuchsin, 0.5 to 1 cc.; glacial acetic acid, 4 cc.
   c. Phenol, 15 cc.; acid fuchsin, 0.5 to 1 cc.; glacial acetic acid, 4 cc.; ferric chloride, (30% aqueous) 2 cc.
   d. Phenol, 20 cc.; phloxine, 10 cc.; NH_4OH (conc.) to slight alkalinity.
   e. Phenol, 20 cc.; rose bengal, 10 cc.; NH_4OH (conc.) to slight alkalinity.

For algae use *a* to *e*, for fungi *a* to *c*, and for root tips, epidermis or *Elodea* leaves, *b* or *c*. Mount *a* to *c* in lacto-phenol plus 20% glacial acetic acid; *d* and *e* in 50% glycerin.

4. *Carbol-hematoxylin.*
   a. Water, 95 cc.; hematoxylin, 1 g.; phenol, 5 g. Add the hematoxylin to the water, bring slowly to boiling with occasional shaking; add the phenol and cool.[2]
   b. Carbol-hematoxylin, 20 cc.; HCl (conc.), 1 cc.
   c. Carbol-hematoxylin, 20 cc.; HCl (conc.), 1 cc.; $FeCl_3$ (30% aqueous), 0.5 cc.

For algae, some fungi, epidermis, root tips, *Elodea* leaves, pollen mother cells, etc. Mount in lacto-phenol made slightly alkaline with concentrated NH_4OH, or in phenol-glycerin (1 *b*.).

Procedures with Lacto-Phenol-Dye (2) or Phenol-Dye (3) Mixtures

Material may be mounted and stained without fixing, but fixation for 1 to 12 hours in formol-acetic (formaldehyde, 10 cc.; glacial acetic acid, 5 cc.; water, 85 cc.) is generally preferable, excepting for most fungi. After fixing wash for a short time in water and transfer the material directly to the dye mixture (2 or 3) in which staining will be rapid. After staining lacto-phenol-dye (2) mounts will often be satisfactory for immediate examination. If staining is deep or one of the phenol-dye (3) mixtures was used, replace it with the appropriate medium, added at one edge of the cover glass as the stain is with-

[2]Shortt, H. E. Rapid method for Heidenhain stain. Indian J. Med. Re. 1923. Cited from Lee's Microtomist's Vade-Mecum, *9th Ed.*, p. 162. 1928.

drawn from the opposite edge; or remove the cover glass, drain, and apply the medium.

Algae such as *Spirogyra* when stained with acid fuchsin, phloxine, or rose bengal show nuclei, chromatophores, pyrenoids (surrounded by shining starch granules) and protoplasmic strands clearly stained. Root tips, especially if stained with acid fuchsin and cotton blue (2 *c.*), show the embryonic region clearly differentiated from the older portion with the nuclei well stained. Leaves of *Elodea* (*waterweed*) stain slowly (6 to 24 hours). Acid fuchsin is recommended for them. The epidermis of *Tradescantia* spp. (spiderwort) or of *Commelina communis* (day-flower) is excellent for mounts. It may be removed from the leaves in pieces a centimeter square. If only a little dye is used the nuclei will be deeply colored in lightly stained cells.

Procedures with Carbol-Hematoxylin (4)

Staining with hematoxylin may be preferable to that with other dyes, especially for nuclei. While it may be omitted, fixing for fifteen minutes to several hours in a chrom-acetic solution (chromic acid, 0.7 g.; glacial acetic acid, 0.5 cc.; water, 100 cc.) gives better results. After fixation wash at least a short time in water and transfer the material to six drops of carbol-hematoxylin *freshly* mixed with one drop of 30% aqueous $FeCl_3$, the mixing being done with a glass rod on the slide; or else transfer to acidulated carbol-hematoxylin containing $FeCl_3$ (4 *c.*). Stain 1 to 3 minutes, wash in water, and mount in slightly alkaline lacto-phenol or in phenol-glycerin. (Slides prepared in this way are usually best after a day and sometimes fade, but may be restained.) Instead of mixing the carbol-hematoxylin with the $FeCl_3$ the latter may be added to the fixing solution (1 cc. to 10 to 15 cc.). Then stain in carbol-hematoxylin or in carbol-hematoxylin acidulated with HCl (4 *b.*) and mount as above.

The mixture of carbol-hematoxylin and ferric chloride is stable for only a few minutes, so should be prepared *just before using*. It stains deeply and quickly. If staining is too deep, a brief washing in 2 to 4% $FeCl_3$ will reduce it.

COMPARATIVE STUDY OF DEHYDRATION

THELMA T. BAIRD,[1] *Dept. of Anatomy, Ohio State University, Columbus, Ohio*

ABSTRACT.—**The paraffin method has frequently been criticised because of its hardening and shrinking effect on tissue. The author believes this distortion is due to the dehydration and not to the immersion in melted paraffin. An experimentally controlled series of various tissues was dehydrated in different dehydrating reagents, dioxan, iso-butyl alcohol, and ethyl alcohol with chloroform. Except for the dehydration, the tissues were treated identically. In every case, dioxan proved to be a better dehydrating reagent with less shrinkage and brittleness than any of the others. Ethyl alcohol with chloroform produced the greatest degree of distortion.**

INTRODUCTION

Within recent years, numerous suggestions have been made concerning the ethyl-alcohol-xylene series in the paraffin method. The chief criticism of this series has been of its hardening effect on the tissues. Hence, the search for some reagent which would satisfactorily replace the ethyl-alcohol-xylene series without its disadvantages.

Larbaud (1921) proposed the mixture of ethyl and *n*-butyl alcohol, which later was recommended by Zirkle (1930). Painter (1924) substituted anilin oil for the higher concentrations of ethyl alcohol. Sheridan (1929) proposed the use of *n*-propyl alcohol; Bradbury (1931) the use of iso-propyl. Margolena (1932) and Stiles (1934) obtained excellent results with animal and insect tissues by using *n*-butyl alcohol, but Smith (1931) had little success with it. Hewitt (1931) wrote quite favorably of iso-butyl alcohol. Sass (1932) recommended the substitution of ethyl alcohol with acetone.

Graupner and Weisberger (1931) completely omitted the alcohol series by removing tissues directly from water, or fixative, into dioxan, a reagent which is miscible with water or alcohol and is a solvent of paraffin. The Turtox News (1934) reported satisfactory results with dioxan, altho their limited experimentation was restricted to Zenker-fixed tissues.

Walls (1932) from his success with the hot celloidin technic, postulated that it is the hot paraffin, and not the heat "per se", which shrank and hardened the tissues, but Stiles suggested that it may be a matter of dehydration and clearing and not of paraffin impregnation. McClung (1929) omitted the soft paraffin in the classical alco-

[1]New located at The Biological Supply House, 761 E. 69 Pl., Chicago, Ill.

hol xylene method and passed directly from xylene into hard paraffin, thus shortening the time in melted paraffin to a minimum.

Apparently, no one has reported any extensive comparative study of this problem, or considered the question of relationship between fixation and the subsequent treatment.

Formaldehyde is generally regarded as a rapid penetrator, but as taking a long time to exert its full effects. In the ordinary short fixation, great shrinkage often takes place during dehydration. This may be due to the shrinking of incompletely solidified protein compounds by ethyl alcohol, or to the effect of formaldehyde upon the lipides of cell membranes.

Bouin's fluid produces very little shrinkage and distortion in the cellular elements. Furthermore, the tissues maintain a sufficiently soft consistency for easy sectioning. Zenker's fluid, or modifications, constitute the most common fixatives for cytological detail, altho the tissue is often rendered very hard, brittle, and crumbly during after treatment.

Thus, we have represented by the three most common laboratory fixatives, three conditions of tissues; namely, soft consistency at first, but usually shrinking and hardening during dehydration; soft consistency thruout; and a hard, brittle condition from the start. Is there any one method which would satisfactorily prepare all three types of tissues for easy sectioning?

To answer this question, the comparative study of various dehydrating methods was started. The selected methods were fast dioxan, slow dioxan, iso-butyl alcohol (representing the higher alcohols), and the classical ethyl-alcohol-xylene technic. Chloroform, however, was substituted for xylene, as by previous experience chloroform was found to produce less brittleness in tissues and is more easily removed than xylene.

Dioxan (diethyl oxide) is a colorless liquid with a faint odor, a boiling point of 101° C. and melting point about 8° C. Its specific gravity is near that of water (1.0418) and its volatility rather high. It is miscible in all proportions with water and with alcohol. It dissolves paraffin slightly when cold, but quite readily when heated.

Iso-butyl alcohol is a straw colored liquid with a distinct pungent odor, a boiling point of 107° C., a specific gravity below that of water (0.810) and of a rather high volatility. It is miscible with water to the same extent as ethyl acetate and is a solvent of paraffin, particularly when heated. It is toxic internally and may produce headaches when its vapors are inhaled.

Methods

Animal tissue, such as liver, striated muscle, thyroid, and intestine, which usually present technical difficulties, were fixed for 24 hours in 10% formalin, Bouin and Zenker formol. Four tissue blocks from the same organ and the same animal were placed in each fixative. The blocks measured $1 \times 1 \times 0.5$ cm., the average size for histological and pathological preparations. A human colloidal tumor was selected for the thyroid material, while the rest of the tissue was of white rat. A colloidal tumor probably presents more technical difficulty than any other tissue, the colloid being particularly susceptible to hardening, fragmentation and brittleness. After fixation, the tissues were washed in running water, 6 hours for formalin material, 24 hours for Zenker formol, while the Bouin material was placed directly into 70% ethyl alcohol.

The four blocks were then separated, one for each dehydrating process, dehydrated, embedded and sectioned at 10 microns. Representative sections at different levels of each block were mounted and stained simultaneously in the same staining tray with Harris' hematoxylin and eosin. Thus, except for the dehydration process, each section received identical treatment.

The detailed methods are as follows:

A. Fast dioxan: 1) fixation; 2) wash in running water; 3) dioxan, 4 to 6 hours; 4) saturated solution rubber-paraffin[1] in dioxan in oven, overnight; 5) rubber-paraffin, 2 hours; 6) rubber-paraffin, 2 hours; 7) embed.

B. Slow dioxan: 3) 2 parts water + 1 part dioxan, 2 hours; 4) 1 part water + 2 parts dioxan, 2 hours; 5) dioxan, 2 hours; 6) saturated solution rubber-paraffin in dioxan in oven, overnight; 7) rubber-paraffin, 2 hours; 8) rubber-paraffin, 2 hours; 9) embed.

C. Iso-butyl alcohol: 3) 70% alcohol, 6 to 12 hours; 4) 95% alcohol, overnight; 5) iso-butyl alcohol, 2 hours; 6) iso-butyl alcohol, 2 hours; 7) rubber-paraffin, 2 hours; 8) rubber-paraffin, 2 hours; 9) embed.

D. Ethyl-alcohol-chloroform: 3) 70% alcohol, 6 hours; 4) 95% alcohol, overnight; 5) absolute alcohol, 4 hours; 6) absolute alco-

[1]The formula for the rubber-paraffin is as follows:

A) Stock rubber-paraffin, a saturated solution of crude rubber in 53–55° paraffin.

B) Paraffin, 53–55°, 720 g.; stock rubber A, 140 g. in winter or 160 g. in summer: bayberry wax, 80 g.; anilin oil, 5 cc.

hol, 4 hours; 7) chloroform, 4 to 6 hours; 8) chloroform-paraffin, overnight; 9) soft paraffin, 1 hour; 10) soft paraffin, 1 hour; 11) rubber-paraffin, 1 hour; 12) rubber-paraffin, 1 hour; 13) embed.

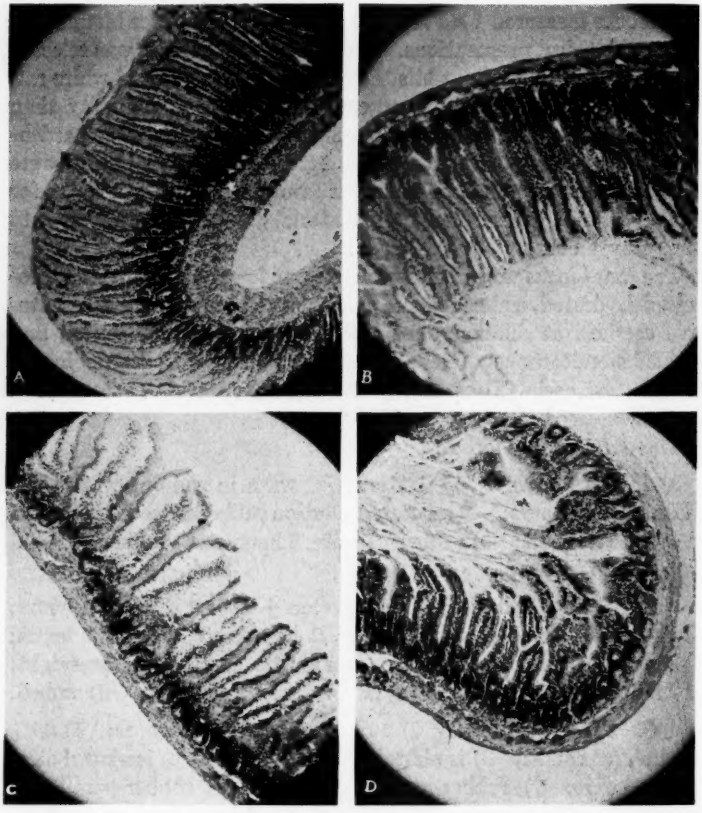


Plate I. Small intestine of white rat, fixed 24 hours in Bouin's fixative, adjacent tissue blocks. The tissue was placed directly into 70% ethyl alcohol after fixation. The blocks were then separated and passed thru the dehydrating reagents; a) fast dioxan; b) slow dioxan; c) iso-butyl alcohol; d) ethyl-alcohol-chloroform.

The Bouin material was placed directly into 70% ethyl alcohol and the dioxan dilutions were made with alcohol instead of water.

Discussion

While dioxan does not completely fulfill the dreams of a microscopist for a perfect dehydrating reagent, yet it is definitely superior to any present known substance (Chart 1). Regardless of the fixation, tissues retained a fairly soft consistency with a minimum of brittle-

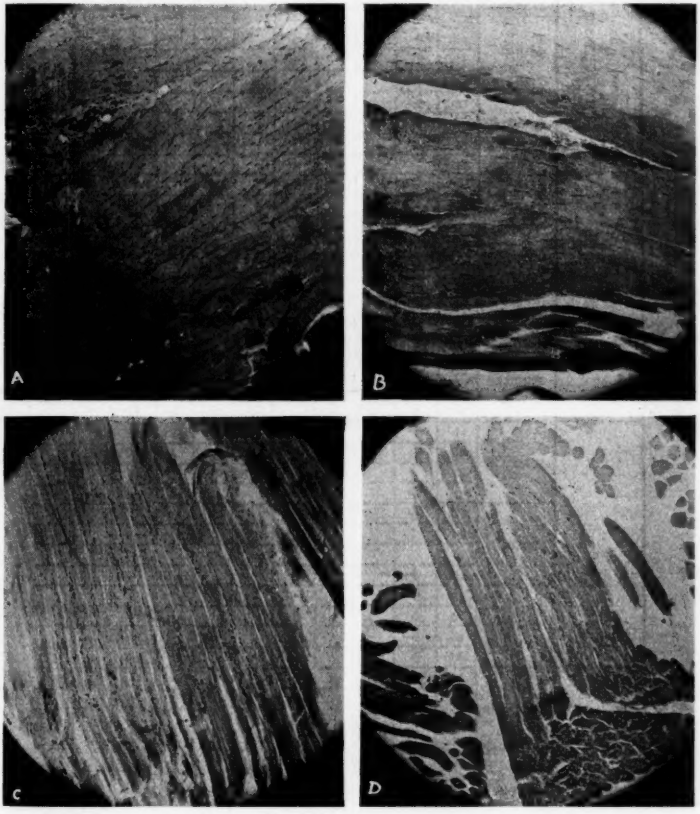


Plate II. Muscle of white rat, fixed 24 hours in 10% formalin, and washed 6 hours in running water, adjacent tissue blocks. The tissue blocks were separated and passed thru the dehydrating reagents; a) fast dioxan; b) slow dioxan; c) iso-butyl alcohol; d) ethyl-alcohol-chloroform.

ness and distortion. The experimental use of different fixatives, adjacent tissue blocks from the same organ and same animal, and simultaneous staining, reduced fixation and tissue variation to a

TISSUE	FIXATIVE	DEHYDRATING REAGENTS	IMPREGNATION			SECTIONS				MICRO-EXAM.	
			GOOD	POOR	INCOMPLETE	GOOD	POOR	SHATTERS	RASPS	SHRINKAGE	SHATTERING
INTESTINE	FORMALIN	FD	+			+				++	
		SD	+			+				++	
		IB	+			+				+++	
		AC	+			+				+++	
	BOUIN	FD	+			+					
		SD	+			+				+	
		IB	+			+				+	
		AC	+			+				++	+
	ZENKER-FORMOL	FD	+			+				+	
		SD	+			+				+	
		IB			+	+				+	+
		AC			+	+				++	+
MUSCLE	FORMALIN	FD	+			+		SL.			
		SD	+					+			
		IB		+	+		+	+	+	+	+
		AC	+				+	++	+	++	+
	BOUIN	FD	+			+					
		SD	+			+					
		IB	+		+	+				+	
		AC	+			+				+	+
	ZENKER-FORMOL	FD	+				+			+	
		SD	+					SL.		+	+
		IB			+			+	+	++	+
		AC	+				+	++	+	++	++
LIVER	FORMALIN	FD	+			+				+	+
		SD	+			+				+	
		IB			+					+	
		AC	+			+		+	+	++	+
	BOUIN	FD	+			+					
		SD	+			+					
		IB			+	+				+	
		AC	+			+				++	
	ZENKER-FORMOL	FD	+			+				+	
		SD	+			+				+	
		IB	+			+				+	+
		AC	+			+			+	++	+
THYROID COLLOIDAL TUMOR	FORMALIN	FD	+		+	+			+	+	+
		SD	+			+				+	+
		IB	+		+	+				++	++
		AC		+			+	+	+	++	+++
	BOUIN	FD	+		+	+			+	++	+
		SD	+			+				++	
		IB	+		+	+				++	++
		AC	+		+	+		+	+	++	+++
	ZENKER-FORMOL	FD	+			+				+	+
		SD	+			+				+	
		IB	+			+				++	SL.
		AC	+			+		+	+	++	+++

Chart 1. Chart of experimentally controlled series of animal tissue. The tissues in all cases received identical treatment except for the dehydration process. Representative 10μ sections were made at different levels thruout the block and were stained by the routine hematoxylin and eosin method.

Abbreviations: fd. fast dioxan; sd. slow dioxan; ib. iso-butyl alcohol; ac. ethyl-alcohol-chloroform; sl. slightly.

minimum. Thus with all extraneous factors controlled, one feels fairly assured that the differences produced are the results of dehydration. As a check upon this assumption, frozen sections of the fixed tissue were made, thus presenting as nearly a normal condition of fixation as possible.

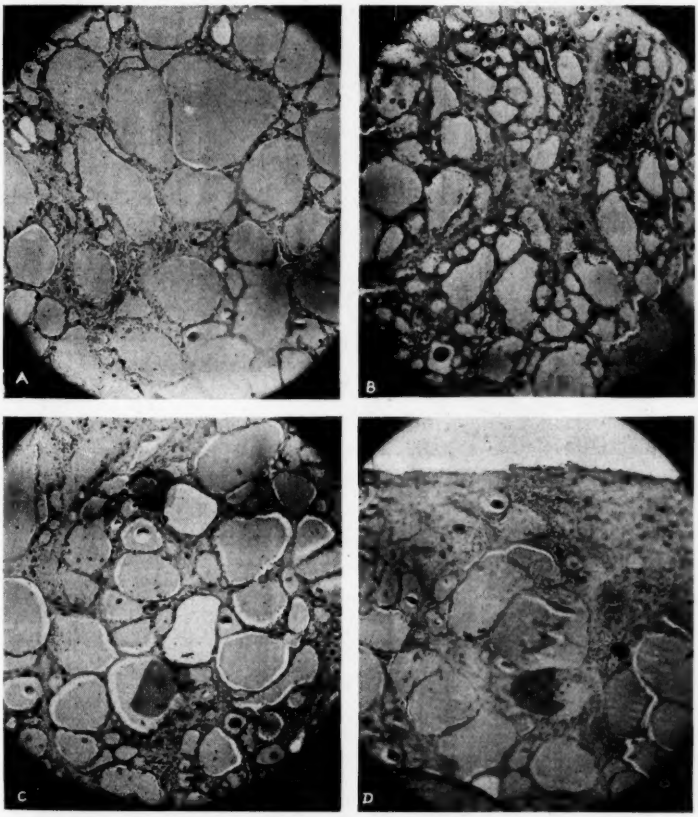


Plate III. Colloid tumor of human thyroid, fixed 24 hours in Zenker formol, washed for 24 hours in running water, adjacent tissue blocks were made. The tissue blocks were separated and passed thru the dehydrating reagent; a) fast dioxan; b) slow dioxan; c) iso-butyl alcohol; d) ethyl-alcohol-chloroform.

The greatest individual variation appeared in the Bouin fixed preparations of small intestine (Plate I). The fast dioxan method alone preserved the integrity of the villi and muscularis. Iso-butyl alcohol produced the greatest amount of distortion, the villi being shortened,

swollen, and the lamina propria and epithelium torn apart. Ethyl-alcohol-chloroform preparation showed short, thickened villi with shattering of the epithelium.

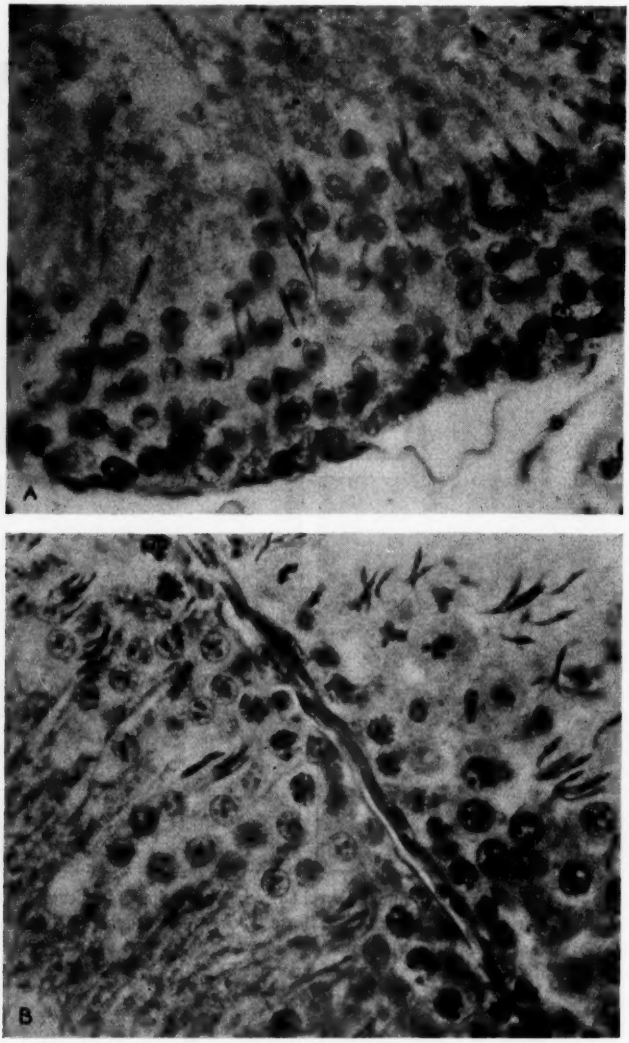


Plate IV. Testis of white rat fixed for 24 hours in Bouin's fixative hemisphered and placed directly into dioxan with several changes. After one month the tissue was removed from dioxan and one piece (a) was embedded with the usual dioxan method. The other piece of testis (b) was left in melted paraffin 9 days before embedding.

Formalin fixed muscle (Plate II) showed a definite shrinking of the muscle fibers in iso-butyl alcohol and a shattering in ethyl-alcohol-chloroform. The Zenker formol thyroid tumor showed similar changes, great shrinkage with iso-butyl alcohol and a shattering with ethyl-alcohol-chloroform (Plate III).

On the whole, the results of the experiment point to the greatest degree of tissue distortion in dehydration with ethyl-alcohol-chloroform, particularly following formal infixation (Chart 1). This suggests that while formalin may congeal or solidify the various cellular proteins, yet it does not render them unchangeable to subsequent treatment, and of the various dehydrating reagents dioxan produced the least amount of change. Zenker-formol fixed tissue appeared to be less subject to change during after treatment. The tissue however, did seem to be rendered less friable in dioxan than with other methods.

The fast dioxan method appeared to hold no particular advantage over the slow method, altho in the case of the formalin-fixed liver and the colloidal thyroid tumor some brittleness was produced by the fast method which was not true of the slow. As there is little time difference between the two methods, the author is inclined to favor the slower, or indirect method.

To determine the permissible variation of time, a rat testis[2] was fixed for 24 hours in Bouin's fixative, then hemisphered and placed directly into dioxan. The material was left in dioxan for one month with several changes, after which one piece was embedded in the usual manner and the other was left *in melted paraffin* at 60° C. for 9 days and then embedded. Both tissue blocks sectioned nicely with no tearing, shattering, or rasping sound. Microscopical examination revealed no shrinkage or distortion in the cellular elements (Plate IV).

Thus dioxan not only prepares tissues for easy sectioning, but produces no hardening effects before or during paraffin impregnation. It does not shrink the tissue and a wide time variation is permitted.

In addition to the experimental series, other material was treated with dioxan. The most remarkable demonstration of its non-hardening effect was the serial preparation of brains of the salamanders, *Cryptobranchus* and *Amblystoma*. The material was fixed in Cox-Golgi fluid for one month and became so friable that the greatest of care had to be exercised in the exchange of fluids. Following the slow dioxan method, the brains were serially sectioned at 10 microns without losing a section and with a minimum of fragmentation.

[2]The testis was selected for this particular test as it is particularly susceptible to shrinkage and distortion.

The author also experimented with the root tips of *Allium* and found less distortion with the slow dioxan method than with any other. It would be interesting to know if this method could be adapted to plant tissue in general.

Conclusions

1. Dioxan, regardless of the fixative, does not render tissue hard and brittle during the dehydrating process, or during paraffin impregnation.

2. The paraffin method is shortened by some 48 hours without sacrifice of or injury to the tissue.

3. Dioxan presents a wider time variation than hitherto experienced with any other dehydrating reagent.

4. Shrinkage and hardening of tissue, so often attributed to the hot paraffin, is directly related to the method of dehydration and preparation of tissue for the immersion in the melted paraffin rather than to the effect of the paraffin.

REFERENCES

Bradbury, O. C. 1931. New dehydrating agent for histological technique. Science, **74**, 225.

Graupner, V. H., and Weisberger, A. 1931. Über die Verwendung des Dioxan beim Eibetten mikroskopischer Objikte. Zool. Anzeiger, **96**, 204.

Hewitt, W. A. 1931. The use of butyl alcohol in the preparation of paraffin sections. Arch. Path., **12**, 599.

Larbaud, Mlle. 1921. Nouvelle technique pour les inclusions et les préparations microscopiques des tissues végétaux et animaux, Compt. Rend. Acad. Sci., Paris, **172**, 1317.

Margolena, L. A. 1932. Concerning dehydrating agents for paraffin embedding. Stain Techn., **7**, 25.

McClung, C. E. 1929. Handbook of Microscopical Technique, Hoeber, New York.

Painter, W. S. 1924. A technique for the study of mammalian chromosomes, Anat. Record, **27**, 77.

Sass, J. E. 1932. Acetone as a substitute for alcohol in microtechnic. Stain Techn., **7**, 65.

Sheridan, W. F. 1929. Weigert's elastic tissue stain prepared with crystal violet. J. Tech. Meth. & Bull. Int. Assoc. Med. Museums, **12**, 123.

Smith, H. M. 1931. Practical suggestions for histological technique. Turtox News, **9**, No. 12, 99.

Stiles, K. A. 1934. Normal butyl alcohol technic for animal tissues, with special reference to insects. Stain Techn., **9**, 97.

Turtox News. 1934. Dioxan, **12**, No. 5, 162.

Walls, G. L. 1932. The hot celloidin technic for animal tissues. Stain Techn., **7**, 135.

Zirkle, C. 1930. Use of N-butyl alcohol in dehydrating woody tissues for paraffin embedding. Science, **71**, 1830.

HISTOLOGICAL APPLICATIONS OF TANNIC ACID AND FERRIC CHLORIDE[1]

HENRY T. NORTHEN, *Department of Botany, University of California, Berkeley, California*

By using tannic acid and ferric chloride in conjunction with a cytoplasmic, cellulose-wall stain (light green or crystal violet) and with a nuclear, lignin stain (safranin), clear preparations of stems of various ages of *Pyrus sp.* (pear) and *Sambucus sp.* (elderberry) and of *Eichhornia sp.* (water hyacinth) root tips showing stages in the development of lateral roots, have been obtained. Such triple stained preparations surpass those stained by the usual combinations of safranin-light-green or safranin-crystal-violet in that the walls of cambial cells and meristematic cells of the growing points as well as young phloem and tracheary elements are more distinctly and permanently stained.

If, as in sections of *Pyrus* stems, the tissues concerned hold the safranin well in the subsequent dehydration, the delicate-walled cells may be stained with the tannic-acid-ferric-chloride combination during the process of dehydration following the staining with safranin. The following schedule has given good results with *Pyrus* sections fixed in forma'in-acetic-acid-alcohol and cut at 12 μ:

(1) Transfer the slide from 50% alcohol to a 1% solution of safranin O in 50% alcohol. Stain 24 hours.

(2) Rinse slide for 10 seconds in 50% alcohol.

(3) Place slide in a 0.5% alcoholic solution of tannic acid (0.5 g. of tannic acid, C. P., Merck or Mallinckrodt, to 100 cc. of 50% alcohol) for 15 to 30 seconds.

(4) Pass the slide thru two Coplin jars of 70% alcohol leaving about 10 to 15 seconds in each.

(5) Place slide in a 1% solution of ferric chloride in 70% alcohol (1 g. of ferric chloride in 100 cc. of 70% alcohol) for 10 to 20 seconds.

(6) Continue the dehydration by leaving the slide in 80% alcohol for 10 seconds, in 95% alcohol for 20 seconds, and in 100% alcohol for 30 to 40 seconds.

(7) Transfer the slide from 100% alcohol to a 0.5% solution of crystal violet (gentian violet, Grübler, uncertified) in clove oil. Stain for 30 to 60 seconds.

(8) Rinse in xylol, transfer to clean xylol, and mount in balsam.

(9) Examine the finished preparation. The parenchyma and collenchyma cell walls should be stained purple, the walls of fibers red or purple, the lignified walls of the xylem red, the cambial cell

[1]See Foster, A. S. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. Stain Techn., 9, 91–2. 1934.

walls black, the nucleoli red, and the cytoplasm purple. If too much safranin has been washed out allow less time in the alcohols; if not enough has been washed out allow more time in the alcohols or add a drop of HCl to one of the jars of 70% alchol.

If the safranin washes out easily, it is desirable to stain with the tannic-acid-ferric-chloride combination before staining with safranin. The following schedule has proven very satisfactory for cross sections of *Sambucus*, for cross and longitudinal sections of *Eichhornia* root tips, and for embryos of *Capsella:*

(1) Transfer the slide from 70% alcohol to a 1% solution of tannic acid in 50% alcohol, and leave for 2 to 3 minutes.

(2) Rinse for 15 to 20 seconds in each of two jars of 50% alcohol.

(3) Place the slide in a 3% solution of ferric chloride in 50% alcohol for 30 to 60 seconds. The ferric chloride solution will blacken as the slides are passed thru it but this will not affect the staining. About 50 slides can be passed thru the alcohols and thru the ferric chloride.

(4) Rinse the slide in 50% alcohol and then transfer to a 1% solution of safranin in 50% alcohol. Stain for 24 hours.

(5) Rinse in 50% alcohol.

(6) Partially destain in 70% alcohol but leave in more safranin than is desired in the finished preparation because the safranin will wash out in the other alcohols and will tend to become reduced in the light green.

(7) Continue the dehydration in 80, 95, and 100% alcohols, leaving the slide in each about 10 to 30 seconds.

(8) Transfer the slide from 100% alcohol to a 0.5 solution of light green in clove oil to which 7 cc. of 100% alcohol has been added for each 100 cc. of clove oil. Stain for 1 to 3 minutes.

(9) Rinse the slide in 100% alcohol; transfer to xylol; and mount in balsam.

Sambucus stems stained according to the above method will have a pleasing appearance and all of the cell walls, including those of the cells of lenticels, will be distinct and clear. The cambial cell walls and parenchyma cell walls will be stained black, lignified walls will be red, and walls of the collenchyma cells will be a blackish red. The nuclei will be red, while the cytoplasm will stain bluish green.

Altho in the *Eichhornia* root sections the parenchyma as well as the meristem cell walls are stained black, the young lateral roots will stand out prominently because the cytoplasm of meristematic cells stains red whereas the cytoplasm of the parenchyma cells stains green.

Heartiest thanks are due to Dr. Adriance S. Foster for his constant coöperation and for the suggestion that the tannic acid method may be valuable in stem preparations.

NOTES ON TECHNIC

THE VIOLET AGAR REACTION AS A DIFFERENTIAL CHARACTERISTIC OF THE *Micrococcus catarrhalis* GROUP.—It was shown by Chapman and Berens[1] that the color of growths of staphylococci on crystal violet agar 1:300,000 could be used for differential purposes. When strains belonging to the *Micrococcus catarrhalis* group (gram negative, biscuit-shaped diplococci some of which were chromogenic) were plated on the violet agar medium, color changes similar to those observed with staphylococci were noted. Certain strains produced violet growths, others produced pale growths, while still others produced white growths.

The crystal violet agar used in these experiments had the following composition:—

Bacto-Beef Extract	3	g.
Proteose Peptone, Difco	5	g.
Bacto-Lactose	10	g.
Bacto-Agar	15	g.
Crystal violet (National, cert.) 1.0%[2]	0.33	cc.
Water to make	1000	cc.
Final pH 6.8 ±		

In view of the implications attached to R and S differences and because of the marked dissociation which occurs in this group of organisms, the color of growths on violet agar was compared with the roughness or smoothness of the strains. The latter characteristics were determined by washing off growths from proteose lactose agar (the above medium minus crystal violet) into normal saline and, after allowing them to settle a few hours, classifying them according to the appearance of the suspensions. If the supernatant was clear, the culture was considered rough. If there was an opalescent or turbid supernatant suspension and a granular deposit, the culture was considered intermediate (SR). If the suspension was uniformly turbid, the strain was considered smooth.

Altho many strains were examined, only 34 were tested. The remaining cultures adhered so tenaciously to the proteose lactose agar that they could not be separated. Of the strains used for comparison, 6 produced violet growths, 15 produced pale growths and 13 were white. The correlation was as follows:—

---

[1]Chapman, G. H. and Berens, C. Crystal violet agar as a differential medium for staphylococci. J. Bact., 29, 437. 1935.

[2]The strength was accurately calculated on the basis of dye substance and not on the crude weight of the dye.

Color of growth	Number of strains tested	Dissociants		
		Smooth	Intermediate (SR)	Rough
Violet	6	5	–	1
Pale	15	1	5	9
White	13	–	–	13

These results suggest that the violet agar reaction may be useful for the study of the *M. catarrhalis* GROUP.—GEORGE H. CHAPMAN, Clinical Research Laboratory, 604 Fifth Avenue, New York, N. Y.

A MEANS OF ELIMINATING WRINKLES IN SECTIONS PREPARED BY THE PARAFFIN METHOD.—Difficulty is often experienced in the preparation of paraffin sections, because of wrinkles in the paraffin and in the tissues. Such wrinkles are particularly common in large round or hollow structures, such as intestine, trachea, stomach, etc. In tissues of this sort the usual method of heating the paraffin strip on warm water often fails to remove the wrinkles and allow the material to flatten out on the slide.

By dissecting away the paraffin from the outside of the tissue, while the paraffin section is floating on the water on the slide, the wrinkles will be eliminated; and the section will flatten perfectly against the slide. This operation should be carried out prior to heating the water on the slide, for warm paraffin adheres to the dissecting needles. Furthermore, removal of the paraffin should be done under a low power dissecting microscope, so that the tissue will not be damaged.

Obviously, such a procedure involves delicate and tedious manipulation. For class use in biology courses, however, where only one or two sections need appear on each slide, the preparations are worth the effort expended. Even if serial sections are required, the method may be useful, for the results are perfect.—RUSSELL W. CUMLEY, Dept. of Zoology, Univ. of Texas, Austin, Tex.

LABORATORY HINTS

FROM THE LITERATURE

A department devoted to abstracts of books and papers from other journals dealing with stains and microscopic technic in general

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

BOOKS

BECKER, ELERY R. and ROUNDABUSH, ROBERT L. Brief Directions in Histological Technique. 6 x 9 in., ix + 80pp. Paper. Collegiate Press, Ames, Iowa. 1935. $1.00.

This booklet contains directions in general histological technic intended primarily for use in college classes in zoology and entomology. Special attention is given to the Zenker-paraffin-Delafield method to which a chapter of 20 pages is assigned. The following five chapters of the book deal with alternative technics in fixation, dehydration, embedding, staining, and mounting. A final chapter includes special methods as applied to protozoa, hydra, worms, earthworms, insects, blood, bone, embryos, and the demonstration of chondriosomes and Golgi bodies.

The book is bound in paper cover with spiral back, a method of binding which allows it very conveniently to lie flat on the laboratory table.—*H. J. Conn.*

CAMERON, GLADYS. Essentials of Tissue Culture Technique. Preface by Robert Chambers. Illustrations and chapter on Photomicrography by C. G. Grand. 6 x 9 in., xvi + 135pp. Cloth. 26 illustrations. Index. Bibliographies. Farrar and Rinehart, New York. 1935. $3.00.

This compact volume of 135 pages, the first of its kind appearing in the United States, presents an excellent resume of the most approved methods of tissue culture. The author has gathered a wealth of experiences during her years of training at the Rockefeller Institute and in the laboratory of Dr. R. Chambers.

The text follows the idea expressed in the introductory paragraphs, namely, that "The technique of tissue culture has generally been considered somewhat forbidding, but its underlying principles are really simple and can be readily comprehended." Every step from the consideration given to the space and basic equipment, physiological solutions, embryo extract, etc., to manipulation of tissue culture and histological technic is presented in a clear and thoro manner. The bibliography of 64 titles comprises principally work published in English, altho a few important German and French references are also given.

The appendix on photomicrography by C. G. Grand is thoroly in keeping with the chapters on tissue culture. Mr. Grand fortunately has chosen simplified equipment. He gives ample yet concise directions that should insure successful photomicrographs from the hands of any microscopist who has mastered a few photographic principles.—*J. M. Thuringer.*

THE MICROSCOPE AND OTHER APPARATUS

CRAIG, R. and WILSON, C. A microtome knife holder for safety razor blades. Science, **81**, 404–5. 1935.

Difficulties are commonly experienced in using razor blade holders commercially obtainable. A holder affording satisfactory rigidity and adjustment and proper bevel angle is fully described, which features curved surfaces of bottom and clamping blocks, eccentrically mounted holding pins and cooling arrangement.—*J. A. de Tomasi.*

KOPAC, M. J. Darkfield micromanipulation with an Ultropaque illuminator. Science, **82,** 70. 1935.

For the study of the surface precipitation reaction in Amoeba the procedure used is as follows: Mount the organisms in hanging drop and place on the moist chamber. The needles of the micromanipulator are adjusted in the bright field of the substage lamp and condenser. The work and observations are carried on in the dark field supplied by the "Ultropaque" illuminator (Leitz), consisting of a ring condenser on each objective. This permits arrangement of all degrees of illumination needed.—*J. A. de Tomasi.*

LENHARD, O. Zweimasstab-Mikrometer-Okular. Zts. Wiss. Mikr., **51,** 68–9. 1934.

Within a micrometric ocular, 2 micrometric scales are brought to cross at right angles; one is fast and permits evaluation of distances from the end of an object, the other slides, allowing estimation of width or thickness at any given distance. It is thought of as a great help in following embryological developments.—*J. A. de Tomasi.*

NELSON, I. A. The use of razor blades for tissue sectioning. J. Lab. & Clin. Med., **20,** 956–7. 1935.

A number of makeshifts are illustrated to show that various types of razor blades can be used for microtome work without special clamp holders. For a good support of the cutting edge, a discarded microtome knife or a similarly shaped piece of metal will do. The blade bevel is kept in close contact with the cutting edge of the old knife by means of a glue or cement. Duco Household Cement has proved satisfactory. The use of a couple of magnets is suggested for holding the blades together while the cement is setting.—*J. A. de Tomasi.*

SCHECHTMAN, A. M. A simple device for the rapid observation of objects in lateral and ventral views. Science, **81,** 404. 1935.

A square observation chamber is built in a block of paraffin. The bottom and one of the side walls are supplied by two juxtaposed 90° prisms. Ventral or lateral views, or both, are obtained simultaneously by altering the position of the device with reference to the objective.—*J. A. de Tomasi.*

SCHMIDT, W. J. Ein Dehnungsapparat zum Gebrauch auf dem Mikroskop, hergestellt von E. Leitz in Wetzlar. Zts. Wiss. Mikr., **51,** 54–65. 1934.

An apparatus is described for the study of stretching tissues, gels, etc., consisting of 2 parallel metal blocks between which the object lies. They are pulled apart in synchronous motion by a set of 2 cogwheels. Displacements are given on a calibrated drum, 1 revolution of which corresponds to 1 mm. total displacement of the 2 holding sides. This apparatus can be used on any common type of microscope stage, also immersed in liquids for work on imbibition.—*J. A. de Tomasi.*

SCHONTEN, S. L. Untersuchungen mit den Mikromanipulator. Arch. exp. Zellforschung, **17,** 429. 1935.

The author presents methods for isolated spores, bacteria, protozoa, etc., and describes growth changes in hanging drops. The technic described refers to his manipulator which he first devised in 1907 with improvements added principally in 1914 and subsequently.—*R. Chambers.*

SCOTT, J. A. An illuminator for the binocular dissecting microscope. Science, **81,** 595–6. 1935.

Especially in the examination of nematode larvae within water drops, it has been noticed that illumination in the orthodox way is not equally distributed to both eyes. This device is designed to correct such condition. Two bulbs, whose distance apart can be regulated, send their respective light beams thru two 500 cc. flasks of alcoholic $CuSO_4$ soln. These may be adjusted so as to give the two beams a correct angle at the microscope mirror. The light then passes a ground glass and a window of plain glass. In assembling the outfit in a light-tight box, the flat side of the bulbs' filament should face the flasks.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

CARLSON, J. G. A rapid method for removing cover glasses of microscope slides. Science, **81**, 365. 1935.

A few hours immersion in a mixture of 90 parts xylene and 10 parts *n*-butyl alcohol will loosen up any cover slip. Anilin dyes, however, will also be taken off.—*J. A. de Tomasi.*

CROSSMON, G. A paraffin block cooler for use with the microtome. Science, **81**, 466–7. 1935.

In order to obtain paraffin ribbons that show little or no compression, a stream of cold air is directed at the block on the microtome. This is accomplished by passing compressed air thur a $CaCl_2$ tube and a copper coil enclosed in a small cooling chamber. This is filled with ice or a freezing mixture. The cold air outlet is kept at a distance proportionate to the amount of cooling wanted. A few sections are cut previous to chilling so as to establish a lead for the ribbon. The air draft does not disturb and no electrification has been noticed.—*J. A. de Tomasi.*

FAY, A. C. A generally applicable method for the enumeration of microscopic objects. J. Lab. & Clin. Med., **20**, 1088–9. 1935.

Method is as follows: Place 0.1 ml. or 0.1 g. of the specimen on a 3 x 1 in. chemically clean glass slide. If necessary a few drops of water or other liquid may be added for spreading. Any objective can be used, provided the proper factor is used in the final evaluation. Factors are given in 2 tables. With aid of a stage micrometer, record the tube length which will give diameters of 1.57, 0.351, and 0.157 mm. for the fields of the low powers, high dry, and oil immersion objectives. The areas represent 1/1,000, 1/20,000 and 1/100,000 of the area of the slide, respectively. If 0.1 ml. of the specimen is employed, the average number of objects per field multiplied by 10,000, 200,000, or 1,000,000 will give the count per ml. Other basic measurements and factors are given for counts based on continuous strip readings.—*J. A. de Tomasi.*

JOHANSEN, D. A. Dehydration and infiltration. Science, **82**, 253–4. 1935.

Successful dehydration and infiltration are not dependent upon successful fixation: the two processes are mutually exclusive. It is assumed that good infiltration occurs when the water is replaced without the water-absorbing capacity of the tissue being destroyed. A dehydrating medium, therefore, should mix in all proportions with water, ethyl alc., paraffin and balsam. These requirements eliminate practically all fluids in common use. Normal butyl alc. is also one of them since with many plant tissues it has been proven to carry about desiccation. Dioxane and tertiary butyl alc. prove to be satisfactory; they give perfect preservation of the image, remove free water but leave the water-absorbing capacity unimpaired, produce no hardening, and do not affect staining properties.—*J. A. de Tomasi.*

NELSON, I. A. The use of proteins for embedding small tissues for sectioning by the freezing method. J. Lab. & Clin. Med., **20**, 964–8. 1935.

This is not an impregnating method and applies chiefly to tissues which hold together within themselves. It is based upon the use of permeable cellophane shaped into an embedding box and acting as a semi-permeable dialyzer. Blocks are formed in such a box by the coagulation of blood or egg proteins. Oxalated whole blood left over from clinical tests and containing at least 14 g. of hemoglobin per 100 cc. may be used. It is emulsified by addition of a small bit of saponin powder. When the bloods from various groups are pooled, it is possibly better to lake the cells of each blood before adding them to the pool. Separate and combine all the blood plasmas from their cells, lake and pool these and then mix equal parts of this lake with the combined plasma. Whole raw eggs may be used instead of blood and merely require stirring of the yolk into the albumin. The procedure of blocking is as follows: Place the cellophane box with the tissue particles in a small dish. Pour the emulsoid protein into the box. Stir and orient the pieces. Pour into the dish enough 20–40% formalin, neutral or slightly alkaline, to fill it up to a little below the level of the emulsoid within the box and let it stand for 2–4 hr. Peel off the cellophane, rinse the block in running water and cut it on the freezing microtome.—*J. A. de Tomasi.*

STEWART, J. D. and MENNE, F. R. A method of staining amyloid in permanent gross specimens. J. Tech. Meth. & Bull. Int. Assoc. Med. Museums, **13**, 60–1. 1934.

Spleen portions preserved in 2% Lugol's soln. and 2% H_2SO_4 give the best results: the amyloid stains mahogany-brown, the surrounding tissues light yellow. Use at least 10 times as much fluid as the volume of the tissue. The tissue should be well fixed in formalin to prevent turbidity in the preserving liquid.—*J. A. de Tomasi.*

WILSON, C. and HOCKADAY, J. S. An easy way to reduce electrification of paraffin ribbons. Science, **82**, 306. 1935.

Static electricity generated at the knife edge can be eliminated from paraffin sections by allowing leakage of the charge into the air. Boiling water present in a sectioning room will furnish sufficiently high humidity to permit cutting ribbons as thin as 2μ in any weather.—*J. A. de Tomasi.*

WYCKOFF, R. W. G. Ultraviolet microscopy as a means of studying cell structure. Cold Spring H. Symp. Quant. Biol., **2**, 39–44. 1934.

Ultraviolet microscopy provides a tool capable of solving many problems in cytology. The author discusses the rationale of the technic, with its advantages and limitations. With ultraviolet light one can see things having about half the diameter of the smallest object discernible with visible light; structures in living cells that are invisible in ordinary light may also be seen.—*M. W. Jennison.*

ANIMAL MICROTECHNIC

ARNIM, S. S. A method for preparation of serial sections of teeth and surrounding structures of the rat. Anat. Record, **62**, 321–30. 1935.

Material, white rat. Best fixation, formalin-mercuric chloride acetic acid. Store 5–7 days in 10% formalin, wash in running water, dehydrate in abs. alc. and stir continuously in a desiccator. Embed in celloidin. Run blocks down to water and decalcify by means of a 7% aqu. soln. of acetic acid until test of acid soln. with ammonium oxalate gives no ppt. Then dehydrate the blocks with propyl alc. and re-embed, starting with 3% celloidin. Harden, clear and embed in 42° C. paraffin. Cut on sliding microtome 7 μ thick. Mount by special technic using propyl alcohol, and stain with dilute Delafield's hematoxylin and aqu. eosin. Method shows retained debris in sulci and on surfaces of teeth, the enamel lamellae, and outline of enamel rods in serial sections 3–10 μ.—*S. I. Kornhauser.*

BALLOWITZ, E. Zur Fixierung und Konservierung der Spermiozeugmen. Zts. Wiss. Mikr., **51**, 51–3. 1934.

The male ducti deferentes and the female receptacula seminis from freshly killed insects are placed in a 0.75% NaCl soln. The "Spermiozeugmen" are isolated by teasing on a glass slide, and handled by the following technic: Remove the tissue debris and turn the preparation as a hanging drop over a 1–2% osmic acid soln. for 15 min. Stain lightly with eosin and mount in glycerin, or spread the fixed material on a cover slip; air dry and fix to the glass by passing several times thru an alc. flame. Stain such cover slip preparations by letting them swim on top of a dye solution. Rinse quickly in dist. water, air dry and mount in balsam.—*J. A. de Tomasi.*

BAUER, K. Über pathologische Reaktionen im embryonal Organismus nach Einwirkung chemischer und physikalischer Mittel. Virchow's Arch., **294**, 477. 1935.

Material: Chick blastoderms. Fixation: Zenker's fluid or Held's 'green fluid' (chromic acid, formalin and acetic acid). Embedding: celloidin-paraffin. Sections treated by modification of Held's method for staining with molybdenum-hematoxylin. Method as follows: Iron alum (40° C.), 20 min. Dilute molybdenum-hematoxylin (at least 4 months old) at 50° C., several hr. Wash in dist. water. Differentiate in ferrocyanpotassiumborax and wash with dist. water for 1–10 min. Place in 2% uranyl acetate, 30 min. Rinse in dist. water. Graded alc. Treat 5 min. in the following soln: Carboxylol, abs. alc., and acetone, to which has been added several drops of alcoholic erythrosin. Results: cytoplasm, blue; intracellular and intercellular substances, red.

An additional method for uranhematoxylin is given: Composition: hematoxylin, 1 part; 70% alc., 200 parts; uranyl nitrate, 5 parts. Place sections in this soln. for several hr. or days at room temp. Wash and differentiate in 2% alum. Wash in dist. water 5–10 min. and differentiate in 2% uranyl acetate for 30 min. Alcohols, xylol, balsam. Results: cytoplasm, blue; intercellular substances blue (depth depending upon treatment with alum).—*H. E. Jordan.*

CASATI, A. Ricerche sperimentali intorno all'azione dei Raggi X sul midollo osseo. Radiobiologia, **1**, 91–5. 1935.

Two groups of rabbits are X-rayed twice at 15-day intervals by irradiations of 8 H. or 400 r. using 140 KW. and 2.5 mA at a distance of 40 cm. with a 0.5 mm. Zn and 2 mm. Al filter. The first group, every other day after irradiation, is given 8 injections (3 cc. each) of trypan blue in 1% NaCl; the second is injected, then irradiated. After 30 days, tissue pieces are fixed in formalin, embedded in paraffin, cut and stained with eosin. In both groups the bone marrow "transitional" cells show the action of irradiation. These forms, however, do not take the vital stain, are considered derived from myoblasts, and not a part of the reticulo-histocytic system. Reticular cells are normal, some of them presenting trypan blue granules deposited on the fibrils.—*J. A. de Tomasi.*

CHAMBERS, R. Disposal of dyes by proximal tubule cells of chick mesonephros in tissue culture. Proc. Soc. Exp. Biol. & Med., **32**, 1199–1200. 1935.

Aqueous solutions of dyes in various concentrations were mixed with the usual tissue culture medium in which were planted fragments of functioning mesonephros of a 9–11-day chick. Isolated segments of the tubules regenerate their cut ends and become converted into closed sacs into which progressive accumulation of color can be observed. Dyes used were: neutral red, C. I. No. 825; Nile blue sulfate, C. I. No. 913; xylene cyanol FF, C. I. No. 715; amaranth, C. I. No. 184; acid fuchsin, C. I. No. 692; orange G, C. I. No. 27. Dyes appear to be taken up by proximal tubules either by passive infiltration of a lipoid soluble dye regardless of temperature, within viable limits, or by an infiltration dependent on the metabolic activity of the cells. Sulfonated dyes actively taken up by renal cells could be arranged in series, at one end of which were dyes which passed into the lumina of the tubules directly, and at the other end of which were dyes collected in segregation vacuoles in the cells with little tendency to pass into the lumina of the tubule.—*M. S. Marshall.*

DAVID, L. T. A method of preparing paraffin sections of bone. Science, **82**, 179. 1935.

Pieces of bone as large as 2 cm. × 2.5 cm. × 2 mm. can be used. Decalcify with 5 to 10% HNO_3 followed by 5% aq. Na_2SO_4 sol. (24 hr.) and running water (24 hr.). Dehydrate, clear in 1:1 abs. alc. and chloroform, 2 changes of chloroform; pass thru several changes of paraffin (M. P. 47° to 49°), embed in harder paraffin (M. P. 56° to 58°). Expose the side to be sectioned to water until the tissue shows definite swelling (2–10 days); dry the surface and dip in melted paraffin. Flood clean slides with the following fixative: Water 100 cc., standard water-glass sol. 1 cc., concd. NH_4OH, 1 cc. Add sections, warm slightly, drain excess fluid. Dry slides at least 48 hrs. Good results have been obtained with small bones of various structure and hardness; the staining calls for hemalum and eosin.—*J. A. de Tomasi.*

FAY, A. C. The detection of formaldehyde in milk by means of the methylene blue reduction test. J. Dairy Sci., **18**, 327–31. 1935.

Confusion has frequently arisen from analyses of milk samples which showed a short reduction period (less than 30 min.) and at the same time a low plate count. Since in such cases tests for formaldehyde always proved positive, the methylene blue procedure might help in locating cases of adulteration with this preservative. Serial concentrations of HCHO from 1:100 to 1:100,000 were made up in milk which was plated out immediately, and after 24 hr. at 21° C. Duplicate controls and also methylene blue reduction tests were set up. Milk alone reduced the dye in 660 min.; a concentration of 1:100,000 of the preservative cut the time down to 259 min.; 1:1000 to 21 min. Higher concentrations increasingly inhibited reduction. The range of dilutions between 1:15,000 to 1:25,000 established close limits: in greater concentrations the preservative is readily tasted; at higher dilutions it is ineffective.—*J. A. de Tomasi.*

GERSONI, C. S. A window technique for fetal observation. Anat. Record, **63**, 169–73. 1935.

Altho the present paper deals only with gross examination, it is of interest to microscopists because window technic is often adapted to the study at close range of histological details of the development of blood vessels, lymphatics, and nerves, and of the action of vital stains in the body.

White rats pregnant two weeks are depilated on the abdominal wall, incised, and a portion of the wall removed. Silk loops are placed anterior and posterior to a single foetus. These loops are drawn thru holes in the anterior and posterior borders of a window consisting of celluloid with a tin frame. The foetus in the uterus is drawn against the inside of the window which is sewed to the belly wall. Collodion makes the joint air tight. The movements of the foetus may now be observed until term is reached. Cutting the loops about the uterus allows the observed foetus to be born with its litter mates.—*S. I. Kornhauser.*

GRADWOHL, R. B. H. A new method of staining blood platelets. J. Amer. Med. Assoc., **105**, 1031. 1935.

The following technic is a modification of the method described by Fonio.

Reagents used: Filtered 14% $MgSO_4$ soln. Methyl alc. (National Aniline and Chemical Co.) for counting blood platelets. Giemsa stain. Neutral dist. water, tested for neutrality as follows: Add 5 cc. of the dist. water to be tested to a few hematoxylin crystals placed in a chemically clean test tube. If water turns pink in 2–5 minutes, it is neutral; if it becomes pink before 1–2 minutes, it is alkaline; if it remains yellow for 5 minutes or longer, it is acid. (To neutralize acid water, use 1% Na or KOH; for alkaline water 1% HCl or acetic acid. Add reagent to water a drop at a time and test with hematoxylin crystals.)

Technic: After producing hyperemia by immersing patient's hand in hot water and drying with sterile gauze, place 1 drop of $MgSO_4$ soln. on the end of the finger and puncture thru the drop. When there is a mixture of about 1 part blood and 9 parts $MgSO_4$, mix with needle point and transfer a drop of mixture to a fat-free slide. Smear with a cover slip as for a blood film. Fix in methyl alc. for 5 min. in a Coplin jar. Wash in neutral dist. water to remove any $MgSO_4$ sol.

Mix 5 cc. of Giemsa stain and 5 cc. of neutral dist. water by shaking *slightly* between each drop. Flood slide with stain for 30 min. Wash in neutral dist. water.

Method of counting: Count all erythrocytes and blood platelets in consecutive fields until 250 erythrocytes have been counted. Calculate the number of blood platelets in 1 cu. mm. of blood by the following formula: Patient's red count $\times$ number of blood platelets counted $\times$ 1/1000 = number of blood platelets in 1 cu. mm. of blood. This method eliminates precipitate common in Fonio's method.—*Elizabeth F. Genung.*

KAISERLING, H. Das Gewebsbild des fieberhaften Rheumatismus. XVII. Mit. Veränderungen der feineren Muskelnerven beim Rheumatismus. Virchow's Arch., **294**, 414. 1935.

In the course of preparing sections by the usual silver impregnation method a solution of dextrose (strength not given) was used in place of formalin as a reducing agent. —*H. E. Jordan.*

KIMURA, R. and MURAKAMI, J. Lymphe als Nährmedium zur Züchtung der Gewebe. Arch. exp. Zellforschung, **17**, 335. 1935.

Lymph is obtained from rabbit of about 3,000 g. weight thru the vas efferens of the popliteal lymph node after having ligated the vas till it swells. One may also obtain lymph from the vasa afferentia which lie under the gland. Within half an hour one is able to obtain 1 cc. lymph. Massage of the peripheral parts of the lower extremities assists the operation.

The lymph coagulates soon after adding tissue extract. No hefarin is necessary. Spleen cultures grow much better than in blood plasma.—*R. Chambers.*

LANDGRAF, G. Beitrag zur Markscheidenfärbung. Zentbl. allg. Path., **63**, 56. 1935.

Application is made of chlorophyl as a myelin sheath stain with a red nuclear counterstain. Dissolve Chlorophyll. spissum (Bayer-Meister-Lucius) 2 g. in 50 cc. of a mixture of 70% alc. and acetone equal parts by heating slowly just to boiling. Filter

after cooling. Use formalin-fixed frozen sections in water; rinse briefly in 75% alc.; chlorophyl soln. 10 min.; rinse in 75% alc.; several min. in dist. water; 2–3 min. in Kernechtrot (100 cc. 5% $Al_2(SO_4)_3$ solution + 0.1 g. Kernechtrot) as counterstain; wash in water; embed in levulose syrup. Myelin sheaths stain dark green, ganglion cells red.—*H. A. Davenport.*

LEWIS, M. R. Effect of the vital dye fluorescent X (reduced neutral red, Clark) on living chick embryo cells in tissue cultures. Arch. exp. Zellforschung, **17**, 96–105. 1935.

Technic: Neutral red was reduced by sodium dithionite; buffered heavily at about pH 4 to 6; separated crystals were washed many times with water; 1 sample was reduced in the presence of a phosphate buffer, the other in the presence of an acetate buffer; recrystallized from methanol and water. Staining reactions of fluorescent X on living tissues usually were made 1:8000 parts soln. of dye in culture media.

Cultures of chicken embryo tissue were prepared in hanging drops of Locke-Lewis soln.; grown at 39° C. for 2–3 days; culture containing dividing cell selected and a cell in mitosis microscopically located; cover glass removed; under surface of growth bathed with a few drops of sterile medium. Cultures were fixed at varying periods by means of Zenker-formol, containing 0.5% acetic acid and later stained with Harris' hematoxylin.

Results: Fluorescent X was taken up by living cells in tissue cultures and accumulated in certain granules as the reduced (yellow) dye. After 24–48 hr. the dye in the stained bodies became oxidized to neutral red, remained red until the cell degenerated, then returned to the reduced (yellow) form.

Abnormal mitosis occurred in the presence of the fluorescent X in the reduced state; abnormalities became fewer as the dye changed from yellow to red; within 48 hr. almost all divisions were again normal.—*Robert Chambers.*

LUDFORD, R. J. The cytological action of methylene blue. Arch. exp. Zellforschung, **17**, 339–59. 1935.

Dyes: "Tabloid methylene blue" of Burroughs Wellcome, Gurr's "methylene blue for injection," and "standard stain" methylene blue of British Drug Houses, Ltd.

Tissue: Cover glass cultures of (a) chick embryo heart in fowl plasma, and in fowl serum, (b) rat embryo heart in rat plasma, and in rat serum, (c) mouse embryo heart in mouse serum, and in rat plasma.

The dye was used in concn. of 1 part in 10,000 parts of Ringer soln. It was applied in each case by raising the cover glass of the culture and filling the well of the hollow glass slide. The culture was resealed and returned to the incubator for 4–8 min. It was then mounted in a drop of the same Ringer methylene blue soln. alone on a glass slide and examined at room temp.

Results: Methylene blue stains the mitochondria, and cytoplasmic vacuoles and granules of living cells. Mitochondrial staining is inhibited by bright light, low temp. and by cyanide. The dye readily penetrates the nucleus and stains the nucleolus followed by coagulation. Dead cells stain diffusely. Both normal and malignant cells react alike to the dye, Janus green, toluidine blue and brilliant cresyl blue act similarly.—*Robert Chambers.*

MANNA, LA. Über Myoblastome. Virchow's Arch., **294**, 663. 1935.

Object: To demonstrate cross striations in tumors of myoblast origin following fixation with Jores' fluid and silver impregnation according to Achucarro (modified by del Rio Hortega). Method: paraffin embedding, thin sections; silver impregnation; dist. water, then stain as follows. Concentrated $FeCl_3$, 20 min.; dist. water, wash until yellow color is gone; stain for 20 min. in soln. made up as follows: 10% iron hematoxylin dissolved in abs. alc. is added in ratio of 1% to dist. water and to 3 cc. of this dilute soln. is added 1 drop of 10% aq. $FeCl_3$. Slides are held horizontally and covered with the stain. Wash with water. Differentiate with following soln: 100 cc. of 10% $FeCl_3$ plus 2 cc. 10% iron hematoxylin. Control differentiation with microscope. Wash with dist. water with slides in vertical position. If cellular elements are overstained, decolorize by allowing a satd. aq. soln. of Li_2CO_3 to flow over sections. Rinse in dist. water, pass thru graded alcohols and xylol and embed in balsam. Results: cross-striations, nuclear structure, intra- and intercellular bile capillaries clearly demonstrated.—*H. E. Jordan.*

MANSTEIN, B. Über die Einwirkung von sauren und alkalischen Lösungen auf Organschnitte und ihre Beziehungen zur Karyolyse. Virchow's Arch., **294**, 120. 1934.

To demonstrate the effect of acid or alkaline solutions on nuclei of different tissues, thin frozen sections cut under aseptic conditions are kept at either 4° C., 20° C. and 37° C. or exposed to buffer solutions of KH_2PO_4 and K_2HPO_4 (pH 5.0, 6.0, 7.0 or 8.0) for 24–48 hr. and stained with Mayer's hemalum.—*H. E. Jordan.*

OKAJIMA, K. Färbung durch Eisenchloridalizarinlack (Omnichrom). Folia Anat. Jap., **13**, 383–4. 1935.

This stain, named "Omnichrom" by the author, is particularly praised for its simplicity of preparation and use. The stain consists of 2% aq. $FeCl_3$, 10 cc.; sat. aq. alizarin red S, 60 cc. Before use dilute with twice as much water. The paraffin, celloidin or other sections pass from water into the stain for 0.5–2 min. Wash in tap water, dehydrate in alc. with care not to overdo. Results: chromatin, collagenous tissue, cell wall and membrane, fibrin, cytoplasm of nerve cells, acidophile and basophile cells stain violet or dark violet; ground mesh of young bone, violet-red; erythrocytes, nuclear bodies, keratin and the lense fibers, orange-red.—*J. A. de Tomasi.*

OKINO, K. Metallimprägnationsversuche an den Erythrocyten. II. Imprägnierung der polychromatischen Erythrocyten bei der experimentellen Phenylhydrazinanämie. Folia Anat. Jap., **13**, 183–8. 1935.

It was previously proved that with normal erythrocytes, the stronger their negative charge, the more silver they take on by impregnation. Polychrome erythrocytes carry a stronger negative charge than orthochrome ones. Will they then also show high affinity for Ag-ions? Rabbits made anemic by phenylhydrazine are the source of polychrome erythrocytes. The air dried blood smears are treated with the usual silver reagent (Okino, K., Folia Anat. Jap., **12**, 373–81. 1934) with a further addition of 25 drops of 2% $AgNo_3$ to 100 cc. of the reducing soln. Giemsa stains serve as controls. Results: Stronger polychrome cells, packed full of very fine dark brown particles, stain uniformly dark brown; light polychrome cells either show brown-yellow particles or scattered precipitates on a colorless background. Orthochrome erythrocytes instead appear quite clear and colorless.—*J. A. de Tomasi.*

PAILLOT, A. Contribution à l'étude des maladies intestinales du ver à soie, deux types nouveaux de dysenterie non infectieuse. Ann. Inst. Pasteur, **54**, 627–48. 1935.

In the study of two new types of intestinal disturbances in the silkworm, the technic employed was as follows: Fix either in saline formol (40% formalin, 20 parts; saline, 80 parts) or in Regaud's bichromate-formol (40% formalin, 20 parts; 3% $K_2Cr_2O_7$, 80 parts). After about three weeks of chromate fixation, embed in paraffin. Stain, according to Kull's method, in warm 20% acid fuchsin in anilin, followed by 0.5% toluidine blue. Differentiate in 0.5% aurantia. Chondriome and nucleoli appear red; chromatin, blue. (No further details of technic given in the paper)—*J. A. de Tomasi.*

RATHMELL, T. K. and JONES, H. W. The preservation of blood films. J. Lab. & Clin. Med., **20**, 954–6. 1935.

The method is intended to be an improvement on the usual Canada balsam technic. The stained dry blood film is covered with a thin coating of lacquer (lacquer No. 14. J. H. Weil Co., 1315 Cherry St., Philadelphia) by means of a No. 16 artist's sable brush. The preparations will be dry in 60–75 sec. and remain impervious to attack by xylol or cedar oil. Under a dry objective the film has an appearance superior to that of the unmounted slide. Under oil immersion, results are equal to such as obtained with conventional mounts. Use a dry slide and apply smoothly at one stroke.—*J. A. de Tomasi.*

SAGEBIEHL, H. Über die Blutversorgung der alternden Herzen. Virchow's Arch., **294**, 147. 1934.

Object: To keep lumens of coronary arteries open for histologic study of character of wall and size of lumen. Healthy hearts removed from body and cleaned, kept for 24 hr. on ice covered with gauze; then placed in incubator at 39° C. for 6 hr. Canula inserted in coronary artery and Ringer's soln. (at 39° C.) injected at pressure of 180 mm. Hg, followed by barium-gelatin (at 39° C.). Heart is then plunged into ice-cold forma-

lin. After fixation for 48 hr. small pieces of heart wall are removed and decalcified in formic acid plus equal parts of 10% formalin (decalcification because hearts were from old people). Pieces are embedded in paraffin, sectioned and stained with Weigert's, hematoxylin-Sudan, or Kernechtrot.—*H. E. Jordan.*

WINKLER, J. E. Über die Beziehungen Zwischen Plasmazellen und Mikrogliazellen in dem nervösen Parenchym der Schlafkranken. Zts. gesam. Neurol. u. Psychiatrie., **153,** 160–4. 1935.

Coincident staining of microglia and plasma cells is described. A) Microglia. Brain formalin fixed, frozen sections, wash 3 times in hot water, then into 0.3% alcoholic soln. *Mastix* for 2–8 hr. Wash 3 times in dist. water; place in 2% HBr 14–24 hr. Wash 3 times in water; place in 2.5% Na_2CO_3 6–24 hr.; impregnate in Hortega's ammoniated silver soln. (diluted 5–8 times) for 0.5–2 min. Rinse quickly and reduce in 20% unneutralized formalin. B) Plasmacells. Transfer sections stained as above to May-Grünwalds stain diluted 6 drops to 60 cc. dist. water, 24 hr. Wash thoroly in dist. water, differentiate in abs. alc. until sections appear pale blue. Creosote-xylene, xylene, balsam. Plasma cells deep blue, microglia brown.—*H. A. Davenport.*

YASUZUMI, G. Über den isoelektrischen Punkt der tierischen Gewebe. IV. Mitteilung. Über den Einfluss von Fixierungsmitteln auf die Färbbarkeit der Erythrozyten. Folia Anat. Jap., **13,** 333–42. 1935.

The influence of fixatives on the stainability of erythrocytes is studied on mouse blood cells. Their isoelectric point is determined colorimetrically by means of the Rovibond tintometer. The influence of H-ion concn. is studied by holding the cells 2–30 min. in isotonic salt soln. at various reactions, followed by alcohol fixation. The pH of 17 different fixatives is determined colorimetrically as well as potentiometrically, whenever possible. The fixatives' capacity to coagulate hemaglobin is tested on the filtrate obtained from the pptn. of the blood stroma by means of the phosphate-citric acid buffer soln.

When erythrocytes treated with salt soln. at various reactions and alcohol-fixed are stained with toluidin blue and also with Ponceau, the color intensity decreases toward the acid range with the basic dye and toward the alkaline with the acid dye. Trichloracetic acid, $K_2Cr_2O_7$, $AgCl_2$, CrO_3, and Muller's and Zenker's fluids, tend to displace the isoelectric point in the direction of alkalinity; picric acid H_2PtCl_6, OsO_4, Helly's, Orth's, Rabl's and all mixed fluids containing OsO_4, shift it toward the acid side.—*J. A. de Tomasi.*

PLANT MICROTECHNIC

BECKER, W. A. and SKUPIENSKI, F. X. Observations protoplasmatiques vitales sur *Basidiobolus ranarum* Eidam. Compt. Rend. Acad. Sci., **200,** 1620–2. 1935.

The fungus *Basidiobolus* was grown on 4% beer wort agar or directly in various concentrations of beer wort. Neutral red, methylene blue and cresyl blue were added to the solid or liquid cultures in concentrations of 1:5,000 or 1:10,000. The growth was normal while the hyphae remained unstained. This is attributed to a decrease in pH of the medium which fell from neutrality to 5.24 in 3 months. Similar results (using neutral red only) were secured by microcultures under a cover slip resting on 4 drops of wax. At higher concentrations of the dye, some structures at first take on the stain, especially the vacuoles, cytoplasmic granulations and sometimes their membranes, but soon the color gradually disappears in definite shapes and the cell resumes further growth activities; the cytoplasm and nucleus migrate to the upper end and develop a transverse wall framing off the rest of the empty mycelium. At times even the nucleolus will temporarily take on the dye.—*J. A. de Tomasi.*

FREY-WYSSLING, A. Über die Verschiebungsfiguren zellulosiger Zellwände. Zts. Wiss. Mikr., **51,** 29–36. 1935.

The origin of displacement patterns in plant cell walls is dependent upon several conditions: membranes must be of pure cellulose and the object must display an ideal fibrous structure, i. e., the orientation of the rows of micellae must be parallel to one another and to the morphological axis of the cell. Displacement patterns may occur not only with bast fibers but also with membranes of other cells like the hourglass cells of leguminous seed shells. These patterns are not to be taken as lattice figures in strict crystallographic sense, but rather as "strains" in their micellar structure.—*J. A. de Tomasi.*

LENOIR, M. Étude des deux variétés de chromatine dans le noyau du *Lilium Martagon*. Compt. Rend. Soc. Biol., **118**, 1554–6. 1935.

Ovaries of *Lilium Martagon* offer good material for the study of two nuclear chromatins, the reticulin and the nucleolin. Fix in Bouin-Duboscq-Brasil (Compt. Rend. Soc. Biol., **103**, 1253, 1930) and stain with the basic fuchsin, malachite green double stain as previously outlined (Compt. Rend. Soc. Biol., **104**, 1282, 1930). The reticular chromatin takes on the fuchsin; the nucleolar, the malachite green. After several years, however, the stain fades, particularly the green.—*J. A. de Tomasi.*

SCHMELZER, W. Bemerkungen zur Anwendung saurer Karminlösungen auf pflanzliche Gewebe. Zts. Wiss. Mikr., **51**, 66–7. 1934.

It is found advantageous to increase the acidity of borax or lithium carmin solutions when intense and durable plant tissue stains are wanted. Add, for example, 1 drop of concn. HCl to 5 cc. of a mixture in equal parts of 70% alc., the carmin soln., and glycerin.—*J. A. de Tomasi.*

SCHMIDT, W. J. Über eigentümliche Veränderungen von Zellulosefäden in Kanadabalsampräparaten. Zts. Wiss. Mikr., **51**, 2–11. 1935.

After several years of inclusion in balsam, cellulose threads from epidermic cells of *Cobaea scandens* seeds undergo considerable changes near the edge of the cover glass. At first some pseudomorphic modifications take place which do not mar the shape of the threads. Later a thinning sets in, accompanied by development of particles and rods of pseudocrystaline nature with a general optical orientation common to the rest of the thread. The positive double refraction of threads shifts gradually to a negative double refraction. It is considered that the balsam as well as O_2 from the air are responsible for these chemical changes comparable to cellulose nitration and acetylation. It is possible, moreover that acid products developing from the neutral balsam may bring about the reaction.—*J. A. de Tomasi.*

MICROÖRGANISMS

CHAPMAN, G. H. and BERENS, C. Crystal violet agar as a differential medium for staphylococci. J. Bact., **29**, 437–48. 1935.

Among other chemical agents, crystal violet (National Aniline, certified) and basic fuchsin (National Aniline, certified) are selected for comparative tests. Dilutions ranging from 1:100 to 1:1,562,000 are measured into sterile tubes in 0.5 cc. quantities. Two strains of staphylococci, one pathogenic and positive to hemolysis and coagulase tests, the other negative, show best differentiation with crystal violet. Proteose lactose agar added with crystal violet in an optimum ratio of 1:300,000 is used for plating. Of the 594 strains tested 51 grow orange, 193 violet colonies and react positively to hemolysis and coagulase tests, while 350 are white and do not correlate. The effect of crystal violet solutions on the color of suspensions of staphylococci is also studied.—*J. A. de Tomasi.*

COWDRY, E. V. and HEIMBURGER, L. F. Morphology of bacilli of rat leprosy. Proc. Soc. Exp. Biol. & Med., **32**, 1422–3. 1935.

It is suggested that the freezing and drying method of fixation of slides preparatory to staining acid-fast organisms in tissues with the Ziehl-Neelsen technic (Gersh, et al.) may contain some unsuspected flaw, or that the bacillus of rat leprosy and the human tubercle bacillus in tissues are less granular than commonly supposed. The usual fixation in 10% formalin in abs. alc. or in Regaud's fluid produces broken organisms whereas the freezing and drying technic yields homogeneous organisms.—*M. S. Marshall.*

DAVID, H. Über ein einfaches und sicheres Verfahren zur Geisseldarstellung. Zentbl. Bakt., I Abt. Orig., **132**, 240–3. 1934.

This method, a combination of those of Gray and Zettnow, is carried out as follows: Inoculate 18–24-hr. agar slants and incubate for 6–8 days. Remove a loopful of bacteria from the upper part of the condensation water and place in a drop of tap water on a glass slide. After 1–2 min., remove a loopful of the slightly turbid drop and transfer to a second drop; 1 to 2 min. later spread a loopful of this drop, snake-fashion, over a flamed cover slip. Air dry and draw rapidly once thru the flame.

Place the cover slip in a fresh mordant soln. (mix 5 cc. 1:10 alum, 2 cc. 1:16 $Hg\,Cl_2$, and 2 cc. 20% tannin) for 10 min., wash under a light stream of water and dry by heating. Transfer to the following silver soln: Dissolve 5 g. $AgNO_3$ in 20 cc. dist. water, add to 6 g. $NaSO_4$ soln. in 10 cc. hot dist. water. Wash the ppt. quickly a few times with 20 cc. dist. water, suspend in 500 cc. water. Mix and store in a brown bottle unshaken. Heat to vapors and remove when the preparation browns or a white precipitate forms around the edge. Place under water and air dry, (not between filter papers). If necessary to bring flagella out more plainly, stain 2 min, with concd. carbol fuchsin. Mount in Canada balsam.—*J. A. de Tomasi.*

De Megni, N. Ricerch sull' agglutinazione aspecifica con sostanze coloranti del gruppo dell' acridina. Boll. 1st. Sieroter. Milan., **13,** 336–43. 1934.

Tripaflavin, chrysanilin, acridin orange and acridin red are used 1:500 in a 0.9% saline soln. at pH 7. In agglutination tubes 0.5 cc. of the culture are added to 0.5 cc. of the dye; the drop agglutination can also be used. Chrysanilin and acridin orange equal tripaflavin in its agglutination power, acridin red sometimes may lyze bacterial cells. "R" strains of the typhoid-coli-dysentery group agglutinate with any of these acridin dyes, "S" strains do not.—*J. A. de Tomasi.*

di Aichelburg, U. L'agglutinazione aspecifica con la fucsina basica nella dissociazione batterica. Giorn. Batter., **14,** 507–12. 1935.

Basic fuchsin in a 1:2000 diln. added to a 1:4000 suspension of bacteria proved to be an unusually sensitive reagent for the detection of bacterial morphological changes. The dye solution agglutinates promptly R type, but none of the S type organisms.—*J. A. de Tomasi.*

Evans, F. L. Note on the fixation of smears for bacteriologic study. J. Lab. & Clin. Med., **20,** 883. 1935.

Some bacterial material does not prove to stick to the glass after usual heat fixation. The author's modification is intended for difficult anaerobic spore-formers. The slide with the smear is held at 37° C. for 30–45 min., transferred for 10 min. to 170° C. in the hot air oven. If organisms are apt to multiply at 37° C., fix first at 55° C. for 15 min.—*J. A. de Tomasi.*

Gehm, H. W. and Heukelekian, H. Eosin methylene blue agar for rapid direct count of *E. coli*. Amer. J. Pub. Health, **25,** 920–3. 1935.

This method has been found to be particularly adaptable to sewage plant control and sewage research. EMB agar is prepared according to the standard method, except that 2% instead of the usual 1.5% agar is used. Plates are poured to a depth of 3 mm. One cc. of inoculum is smeared over the surface and the plate placed in the 37° incubator to dry, with cover removed. The plate is dried for an hour, the inoculum being redistributed over the plate by tilting when about half dry. After the surface is thoroly dry the cover is replaced, the plate inverted, incubated at 37° for 24 hr. and characteristic colonies counted. Close agreement was obtained between this method and brilliant green bile broth tubes on various sewages. The method is cheaper, and quicker than other methods but comparable with them.—*M. W. Jennison.*

Meyer, K. F. and Johnstone, H. G. Laboratory diagnosis of amebiasis. Amer. J. Pub. Health, **23,** 405–14. 1935.

The method of choice in the laboratory diagnosis of amebiasis remains the preparation of wet-fixed, wet-treated, and hematoxylin stained permanent fecal smears. Method: 1. Smear is made with a paste brush on a clean flamed slide. 2. Without allowing slide to dry immerse immediately in the fixing fluid (Schaudinn's). 3. For fixation and staining the following methods should be used: Schaudinn's fluid heated at 60° or over night at room temp., 10 min.; 70% alc. tinged to a wine color with a satd. sol. of iodine in abs. alc. or with Lugol's soln., 10 min.; 70% alc., 5 min.; 50% alc., 5 min.; 35% alc., 5 min.; tap water (running), 2 min.; dist. water-rinse; 2% iron alum-aqueous soln. heated to 30°, 10 min; tap water (running), 10 min.; dist. water-rinse; 0.5% hematoxylin-aqu. soln. heated to 30°, 10 min.; tap water (running), 5 min.; dist. water-rinse; differentiate in 2% iron alum heated to 30°; tap water (running) 20 min. 35% alc., 5 min.; 50% alc., 5 min.; 70% alc., 5 min.; 95% alc., 5 min.; 100% alc.,

5 min.; carbol xylol, 5 min.; xylol, 5 min. Mount with a thin layer of balsam thinned with xylol for temporary slides or with thicker balsam and cover slip for permanent slides.

Schaudinn's fluid. $HgCl_2$, satd. aqu. soln. in normal salt, 2 parts; 95% alc., 1 part. This is the stock soln. and will keep indefinitely. On using add 4 cc. glacial acetic acid to 96 cc. of the stock soln. This soln. should be made up fresh at frequent intervals.

Hematoxylin stain. Stock soln.: hematoxylin, 10% in 100% alc. Cork, and ripen by standing in light for 3 months or more. Stain: hematoxylin, stock soln., 1 part; dist. water, 19 parts.

Iron-alum. Ferric ammonium sulfate crystals, 2% in dist. water. The crystals must be clear and purple or lavender, not yellow.

Carbol xylol may be used in place of abs. alc. if the absolute becomes contaminated with water; or in the handling of a large number of slides it may be used between abs. alc. and pure xylol. (Two washings of toluol may be substituted for the carbol-xylo and xylol.)

Carbol-xylol. Fill wide mouth bottle 1/3 full with clear white crystals of carbolic acid, then fill rest of bottle with xylol. If crystals are yellow or brownish in color the preparation will be discolored.—*M. W. Jennison.*

Monné, L. Permeability of the nuclear membrane to vital stains. Proc. Soc. Exp. Biol. & Med., **32**, 1197–9. 1935.

Vital staining of two Amoeba species was attempted, using some 40 dyes, and a technic of spraying the stain against the nucleus with a micro-pipette. Immersion of the Amoeba into dye solutions failed. In general, the more complicated dyes were more toxic. Vital staining of the nucleus was obtained with: 3 nitro dyes, 7 azo-dyes, 5 thiazins, 4 oxazins, 1 amido-azin, 1 safranin, 1 diamino-triphenylmethane, 4 triamino-triphenyl-methanes, 2 amino-hydroxyl-triphenyl-methanes, 1 pyronin, 1 rhodamin, 3 fluorane derivatives, 4 sulphonphthaleins, 1 acridin. Recovery occurred when the nucleus was partly stained with: water blue, azo blue, methyl blue, alizarin red, and ruthenium red. The following dyes always killed: sodium carminate, trypan red, nigrosin, vital red HR, rosindulin GG and congo red.—*M. S. Marshall.*

Ono, K. Studien über die Färbung der Rekurrensspirochäten, insbesondere über den Zusammenhang der Färbung mit den Vorbehandlungen. Acta dermatol. (Kyoto), **23**, 73–95; **24**, 1–42. 1934. (Abs. in Zentbl. Bakt., I Ref., **119**, 38–40. 1935.)

V. Einfluss der Vorbehandlung mit Kal. permanganat-Lösung auf die Färbbarkeit der Rekurrensspirochäten:

Blood smears from rats infected with relapsing fever spirochetes of Manchurian stock are fixed in 1% $KMnO_4$, thoroly washed, and stained 15 min. in 1% soln. of 282 dyes. Controls (as in all following papers) are fixed 15 min. in alcohol-ether before staining. After $KMnO_4$, erythrocytes stain weaker; spirochetes and leucocytes stronger with basic dyes only; some of the acid dyes act better than others. Best results are obtained with 66 dyes, mostly of the triphenylmethane series, among which are named: ethyl violet, cerise, crystal violet, dahlia, extra violet, fuchsin, gentian violet, indol blue, indophene blue, magenta, marine blue, methyl violet, safranin, thionin, ultra blue and Victoria blue.

VI. Einfluss der Vorbehandlung mit Alaunlösung auf die Färbbarkeit der Rekurrensspirochäten:

Fixation of spirochete blood for 15 min. in 5% alum is compared again with that in alcohol-ether. Erythrocytes stain weaker, spirochetes and leucocytes mostly stronger. Marine blue, methyl violet, new Victoria blue, and Victoria blue are named among the 29 best dyes.

VII. Einfluss der Vorbehandlung mit Karbollösung auf die Färbbarkeit der Rekurrensspirochäten:

Fixation of spirochete blood smears with 5% phenol for 15 min. yields: stronger stain of erythrocytes and blood plasma, weaker stain of spirochetes and erythrocytes. Of the 282 dyes, 45 were satisfactory, e.g., ethyl violet, crystal violet, dahlia, gentian violet, and marine blue. Moreover, 24 dyes stained spirochetes and leucocytes after the phenol but not after alcohol-ether, e. g., acid magenta, benzo blue, benzo purpurin, diamine violet, eosin and patent blue.

VIII. Einfluss der Fixierung der Lösung des molybdänsauren Ammoniums auf die Färbbarkeit der Rekurrensspirochäten:

After 15 min. fixation with 5% $(NH_4)_2MoO_4$, the erythrocytes take a weaker stain, the blood plasma remains colorless, the spirochetes and leucocytes stain stronger. Best suited are 47 dyes among which are named: ethyl violet, "baselin blau", crystal violet, dahlia, gentian violet, magenta, methyl violet, ultra blue, and Victoria blue.

IX. Einfluss der Fixierung mit Kal. bichromat-Lösung auf die Färbbarkeit der Rekurrensspirochäten:

After 15 min. fixation with 5% $K_2Cr_2O_7$ erythrocytes stain weaker, spirochetes and leucocytes much stronger. The blood plasma remains unstained. Among the 26 best suited dyes are: "baselin blau", crystal violet, gentian violet, sky blue, marine blue, methyl violet and Victoria blue.

X. Einfluss der Fixierung mit Müllerscher Flüssigkeit auf die Färbbarkeit der Rekurrensspirochäten:

After 15 min. fixation in Müller's liquid the erythrocytes stain weaker, spirochetes and leucocytes mostly stronger. The plasma does not take the stain. Among the 25 best dyes are: "baselin blau", dahlia, gentian violet, sky blue, marine blue, methyl violet, violet red and Victoria blue.

XI. Einfluss der Fixierung mit dem Orthschen Gemische auf die Färbbarkeit der Rekurrensspirochäten:

After 15 min. fixation in Orth's liquid, results are like those with Müller's fixative. Among the 39 best dyes are: ethyl violet, "baselin blau", crystal violet, dahlia, gentian violet, sky blue, magenta, marine blue, methyl violet, violet red and Victoria blue.

XII. Die Färbemethoden der Rekurrensspirochäten:

Fixation is performed here for 5–15 min. with 10% formalin, or 3% H_2O_2, or 1% $KMnO_4$ (also formalin followed by either of the other two). Staining time is reduced to 2–5 min. (instead of 15 min.) and the conc. to 0.2% (instead of 1%). All 3 fixatives give good results, altho it is hard to decide which to recommend owing to their respective distinct characteristics and advantages. Formalin-fixed smears stain well with ethyl violet, "baselin blau", crystal violet, dahlia, gentian violet, marine blue, methyl violet, and Victoria blue. Fixation in H_2O_2 proves even better, adding to the list several other dyes among which are sky blau and ultra blue. After fixation in $KMnO_4$, staining succeeds with a great number of dyes in 2 min. and with 0.2% soln.—*J. A. de Tomasi.*

PETROFF, S. A. and GUMP, W. S. Bacteriostatic and bactericidal studies of various dyes and allied compounds. J. Lab. & Clin. Med., **20**, 689–98. 1935.

A number of compounds with established bacteriostatic and bactericidal properties have completely failed to bring about desired results when used for therapeutic purposes. Since ordinary beef infusion media are unsuited for such a study, human serum agar was employed. The bacteriostatic property of some 130 dyes and allied compounds were studied on 10 Gram-positive and 7 Gram-negative organisms; the effective ones were selected for bactericidal tests. The list of organic dyes employed includes representatives of nitroso, nitro, azo, stilbene, triphenylmethane, xanthene, acridine, quinoline, azine, oxazine, thiazine and anthraquinone dyes. Plates were poured using pneumococcus agar of pH 7.6, cooled to 45° C. and added up to 10% with pleural fluid. Each Petri dish was divided in 2 halves by means of a strip of cardboard to be withdrawn after the control agar had solidified in one of the compartments. The second compartment received the serum agar plus the dye in dilutions ranging from 25,000 to 200,000 for Gram-positive organisms and from 5,000 to 100,000 for the Gram-negative. The dyes were first dissolved either in water or 95% alc. By means of rulings on the bottom, 6 or 7 streaks were possible across the 2 compartments of each plate, using 0.1 cc. of inoculum from an 18-hr. growth and a 3 mm. platinum loop. Incubation was set at 37.5° C. and results recorded after 48 hr.

Most of tested compounds proved to be of little or no value. The basic triphenylmethane dyes were most effective with the acridine dyes next in order.—*J. A. de Tomasi.*

SANDER, F. Bakterienfärbung durch Kaliumpermanganat; zugleich eine einfache Sporenfärbung. Zts. Hyg. Infektionskr., **116**, 335–6. 1935.

Clean the slide with ether and make a smear from a solid growth suspended in saline or dist. water. Air dry and fix in the flame. Flood for 2 min., preferably with a

cold satd. $KMnO_4$ soln. in 1% H_2SO_4. Rinse, blot dry, and observe in oil. Owing to stronger oxidation of the membrane, bacteria appear stained yellow with a darker outer zone. In case of spores, follow same technic, but heat the oxidant for 2 min. on the slide. Vegetative cells stain yellow; spores, brown.—*J. A. de Tomasi.*

SANDER, F. 50 Jahre Gramsche Färbung und ihre Theorie. Zentbl. Bakt., I Abt. Orig., **133,** 385–400. 1935.

Following an ample review of the many diversified opinions as to the nature of the Gram stain, a broad analysis of conditions affecting the reaction was studied. Organisms of the coccus, bacterium, bacillus type, and yeasts were fixed on the slide by flaming and treated, previous to staining, with a number of reductants and oxidants. The action of hypertonic and hypotonic solutions of neutral salts as well as of non-electrolytes was also considered. Furthermore, suspensions of materials and the influence of the biological polar system of age and environment were similarly taken into consideration. The theory of condensation nuclei for the deposition of the stain was also tested.

Briefly, many reversible reactions were possible whereby Gram-positive organisms may be made Gram-negative and *vice versa*. The theoretical considerations under each of the chapters are developed at great length.—*J. A. de Tomasi.*

SYDENSTRICKER, V. P. and VRYONIS, G. P. Vital staining of malarial parasites. J. Lab. & Clin. Med., **20,** 1094–6. 1935.

It is asserted that any technic that gives good reticulocyte staining will give satisfactory results with malarial parasites. Most satisfactory is the following: Place a small drop, approx. 2 mm., of sat. soln. of brilliant cresyl blue in sterile saline on a chemically clean slide; put a small drop of blood on a coverslip and drop directly on the stain. Ring with soft petrolatum. Staining is almost immediate, the parasites remaining alive for upwards of 2 hr. on the warm stage. Cytoplasm presents a coarsely stippled appearance, pale blue with slightly darker areas; chromatin is dark blue; pigment readily visible.—*J. A. de Tomasi.*

WILLIAMS, J. W. and GREEN, L. III. Effect of dyes on colonies of certain pathogenic fungi. Proc. Soc. Exp. Biol. & Med., **32,** 625–8. 1935.

Microscopic and macroscopic observations were made with 27 fungi on a medium (4% peptone, 1% dextrose, 1.5% agar, pH 5.6) containing alcoholic nigrosin, litmus, eosin Y, and eosin B. To 5 batches of this basic medium were added, respectively: 2% fluorescein (Schultz No. 585, Lot No. 6054), 1% methyl blue (Lot. No. 3421) and 1% eosin Y (Schultz No. 587, Batch NE-7), 0.5% neutral red (Lot No. 7272), 0.5% Janus green (C. I. No. 133), 0.5% Wright's stain (NWr-7). (These dyes were manufactured by the National Analine and Chemical Company.) Growth was in diffused light at room temperature. Colonies showed characteristic colorations, and cellular elements were in some instances superior to specimens stained from growth on ordinary media.—*M. S. Marshall.*